Applicant's docket: ODDY 007

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## **SPECIFICATION**

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### TO ALL WHOM IT MAY CONCERN:

BE IT KNOWN THAT WE, Stephen W. Michnick, a resident of <u>Montreal</u>, <u>Canada</u>, and a citizen of <u>Canada</u>, <u>Marnie L. MacDonald</u>, a resident of <u>Pleasanton</u>, <u>California</u> and citizen of <u>USA</u>; have invented

certain new and useful improvements in

# FRAGMENTS OF FLUORESCENT PROTEINS FOR PROTEIN FRAGMENT COMPLEMENTATION ASSAYS

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# FRAGMENTS OF FLUORESCENT PROTEINS FOR PROTEIN FRAGMENT COMPLEMENTATION ASSAYS

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This application claims the priority benefit under 35 U.S.C. section 119 of U.S. Provisional Patent Application No. 60/461,133 entitled "Fragments of Fluorescent Proteins for Protein Fragment Complementation Assays", filed April 9, 2003, which is in its entirety herein incorporated by reference. This Application is also a continuation-in-part of pending U.S. Application Serial No. 10/353,090 filed January 29, 2003; which application is a continuation of pending U.S. application No. 10/154,758 filed May 24, 2002; which is a continuation of U.S. Serial No. 09/499,464 filed February 7, 2000; and now U.S. Patent No. 6,428,951; which is a continuation of U.S. Serial No. 09/017,412 filed February 2, 1998; and now U.S. Patent No. 6,270,964.

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#### **BACKGROUND OF THE INVENTION**

This invention relates generally to the fields of biology, molecular biology, chemistry and biohemistry. Specifically, the invention is directed to protein-fragment complementation assays (PCAs) based on fluorescent proteins. This invention is directed to methods for the design and creation of suitable fragment pairs, to the compositions of the fragments, and to combinations suitable for PCA. Preferred embodiments include fragments of mutant fluorescent proteins having properties suitable for biotechnology applications.

The growing list of naturally fluorescent, bioluminescent or phosphorescent proteins includes GFP derived from Aequorea Victoria, and a growing number of sequence variants of GFP with useful properties. The list also includes the red fluorescent protein (RFP) derived from

Discosoma; and the kindling fluorescent protein (KFP1) derived from Anemonia. These proteins are autocatalytic enzymes that are all capable of generating highly visible, efficiently emitting internal fluorophores as a result of endo-cyclization of core amino acid residues. Another common feature of the fluorescent proteins is that the signal is stable, species independent, and does not require any substrates or cofactors for the generation of a signal. These fluorescent proteins are remarkably similar structurally allowing similar principles of protein engineering to be applied across species.

The full-length DNA, and corresponding amino acid sequence of one isotype of GFP ("wild-type GFP") is shown in TABLE 1 and has been fully described and characterized (see e.g. Tsien et al., 1998, Ann. Rev. Biochem. 67: 509-44). The intact protein (Figs. 1 and 2B) generates a strong visible absorbance and fluorescence from a p-hydroxybenzylideneimidazolone chromophore, which is generated by cyclization and oxidation of the protein's own Ser-Tyr-Gly sequence at positions 65 to 67. Newly synthesized fluorescent protein polypeptides need to mature properly before emitting fluorescence. The maturation process involves two steps: folding and chromophore formation. First, the protein folds into a native conformation, and then the internal tripeptide cyclizes and is oxidized. In this regard the fluorescent protein is an enzyme which autocatalyzes the cyclization reaction, requiring only molecular oxygen for completion of the reaction.

A variety of useful mutant versions of the full-length, wild-type GFP have been generated and have been termed 'Aequorea fluorescent protein (AFP) variants' or AFPs. These "mutant fluorescent proteins" have proven to have wide applicability for biology and biotechnology applications as a result of their improved spectral properties. Some of the reported GFP variants are shown in Table 2. By conventional usage, the positions of the mutations (as in Table 2 and

throughout this invention) are denoted relative to the sequence of wild-type GFP (Table 1). Many of these AFPs exhibit vastly improved properties over the original wild-type GFP in terms of signal intensity, generating a fluorescence signal 5 to 30 times that of the wild-type protein. The enhanced GFP (EGFP), which is the basis for nearly all biology applications and for mutant fluorescent proteins, has improved codon usage for mammalian cells.

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Starting with GFP, mutations at the site of the chromophore have been created which result in different color variants. Mutations of the side chains in contact with the chromophore have been shown to further enhance protein folding and brightness. Combinations of mutations have been created that have spectral shifts and that fold more rapidly at 37°C, producing brighter signals for cell biology applications. The most common spectral variants include the widely-used yellow (YFP/EYFP), cyan (CFP/ECFP) and BFP variants (R.Y. Tsien, 1998, "The Green Fluorescent Protein", in: Annual Reviews of Biochemistry 67: 509-544).

Additional mutants of GFP have been created with unique properties. These include a 'CGFP' variant with an excitation and emission wavelength intermediate between CFP and EGFP (J. Zhang et al., 2000, "Creating new fluorescent probes for cell biology", Nature Reviews 3: 906-918). The 'citrine' variant of YFP (YFP-Q69M) confers a lower pKa than for previous YFPs, indifference to chloride anion, twice the photostability of previous YFPs, and much better expression at 37C and in organelles (O. Griesbeck et al., 2001, "Reducing the Environmental Sensitivity of Yellow Fluorescent Protein", J. Biol. Chem 276: 29188-29194).

Several versions of YFP have been created using random mutagenesis. These mutant proteins have fluorescence intensities 3-30 times brighter than EYFP. They include the so-called super-EYFP (SEYFP) (EYFP-F64L/M153T/V163A/S175G) and 'Venus' (SEYFP-F46L) (T. Nagai et al., 2002, "A variant of yellow fluorescent protein with fast and efficient maturation for

cell-biological applications", Nature Biotech. 20: 87-90). Venus contains the novel mutation, F46L, which at 37°C greatly accelerates oxidation of the chromophore, the rate-limiting step of mutation. As a result of the additional SEYFP mutations, Venus SEYFP-F46L also folds well and is relatively tolerant of exposure to acidic or high chloride anion concentrations.

A photoactivatable form of GFP named PA-GFP (GFP-V163A/T203H) has been reported that, after intense irradiation with 413-nm light, increases fluorescence 100 times when excited by 488-nm light and remains stable for days under aerobic conditions (G.H. Patterson & J. L.-Schwartz, "A photoactivatable GFP for selective photolabeling of proteins and cells", Science 297: 1873-1877, 2002).

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TABLE 1. Full-length Aequorea GFP nucleic acid sequence (716 bp)(SEQ ID No:1) and corresponding amino acid sequence (238 aa) (SEQ ID No:2). Amino acids are numbered at every 5th position. This sequence is for the wild-type protein. In "enhanced" versions of GFP (EGFP, EYFP, ECFP) a valine residue is inserted after the initiating 5 methionine . The valine becomes amino acid # 2 and the remaining amino acids are shifted accordingly. Descriptions of GFP mutants (as in Table 2 and throughout the specifications) refer to the numbering shown below. Alternative fragmentation sites that are the subject of the present invention are shown at the following regions (underlined): amino acid residues 38-40 (region 1); residues 101-103 (region 2); 10 residues 114-118 (region 3); residues 154-160 (region 4); residues 171-175 (region 5); and residues 188-190 (region 6). The positions of specific amino acid residues are shown for Tyrosine 39 (Y39), Aspartate 102 (D102), Glutamine 157 (Q157), Lysine 158 (K158), Aspartate 173 (D173) and Aspartate 190 (D190). 15 atq agt ctt ttc aaa gga gaa gaa act gga qtt qtc cca att ctt qtt

13	atg Met 1	agt Ser	aaa Lys	gga Gly	gaa Glu 5	gaa Glu	Leu	Phe	act Thr	gga Gly 10	gtt Val	gtc Val	Pro	Ile	Leu 15	gtt Val
20	gaa Glu	tta Leu	gat Asp	ggt Gly 20	gat Asp	gtt Val	aat Asn	Gly aaa	cac His 25	aaa Lys	ttt Phe	tct Ser	gtc Val	agt Ser 30	gga Gly	gag Glu
25	ggt Gly	gaa Glu	ggt Gly 35	gat Asp	gca Ala	aca Tur	tac Tyr Y39	gga Gly 40	aaa Lys	ctt Leu	acc Thr	ctt Leu	aaa Lys 45	ttt Phe	att Ile	tgc Cys
30	act Thr	act Thr 50	gga Gly	aaa Lys	cta Leu	cct Pro	gtt Val 55	cca Pro	tgg · Trp	cca Pro	aca Thr	ctt Leu 60	gtc Val	act Thr	act Thr	ttc Phe
	tct Ser 65	tat Tyr	ggt Gly	gtt Val	caa Gln	tgc Cys 70	ttt Phe	tca Ser	aga Arg	tac Tyr	cca Pro 75	gat Asp	cat His	atg Met	aaa Lys	cgg Arg 80
35	cat His	gac Asp	ttt Phe	ttc Phe	aag Lys 85	agt Ser	gcc Ala	atg Met	ccc Pro	gaa Glu 90	ggt Gly	tat Tyr	gta Val	cag Gln	gaa Glu 95	aga Arg
40	act Thr	ata Ile	ttt Phe	ttc Phe 100	aaa <u>Lys</u>	gat Asp D102	gac <u>Asp</u>	Gly ggg	aac Asn 105	tac Tyr	aag Lys	aca Thr	cgt Arg	gct Ala 110	gaa Glu	gtc Val
45	aag Lys	tut Phe	gaa <u>Glu</u> 115	990 <u>Glv</u> G116	gat Asp	ace <u>Thr</u>	ctt Leu	gtt Val 120	aat Asn	aga Arg	atc Ile	gag Glu	tta Leu 125	aaa Lys	ggt Gly	att Ile
50	gat Asp	ttt Phe 130	aaa Lys	gaa Glu	gat Asp	gga Gly	aac Asn 135	att Ile	ctt Leu	gga Gly	cac His	aaa Lys 140	ttg Leu	gaa Glu	tac Tyr	aac Asn
50	tat Tyr 145	aac Asn	tca Ser	cac His	aat Asn	gta Val 150	tac Tyr	atc Ile	atg Met	gca <u>Ala</u>	gac Asp 155	aaa Lys	caa Gln Q157	aag Lys K158	aat Aan	gga Gly 160
55	atc Ile	aaa Lys	gtt Val	aac Asn	ttc Phe 165	aaa Lys	att Ile	aga Arg	cac His	aac Asn 170	att Ile	gaa Glu	gat Asp D173	gga Gly	age <u>Ser</u> 175	gtt Val
60	caa Gln	cta Leu	gca Ala	gac Asp 180	cat His	tat Tyr	caa Gln	caa Gln	aat Asn 185	act Thr	cca Pro	act	Gly ggc	gat Asp D190	ggc Gly	cct Pro
65	gtc Val	ctt Leu	tta Leu 195	cca Pro	gac Asp	aac Asn	cat His	tac Tyr 200	ctg Leu	tcc Ser	aca Thr	caa Gln	tct Ser 205	gcc Ala	ctt Leu	tcg Ser
70	aaa Lys	gat Asp 210	ccc Pro	aac Asn	gaa Glu	aag Lys	aga Arg 215	gac Asp	cac His	atg Met	gtc Val	ctt Leu 220	ctt Leu	gag Glu	ttt Phe	gta Val
, 0	aca Thr 225	gct Ala	gct Ala	gly aaa	att Ile	aca Thr 230	cat His	ggc Gly	atg Met	gat Asp	gaa Glu 235	cta Leu	tac Tyr	aaa Lys		

Table 2. Spectral cha	aracteristics o	f the major classes of Aequ (AFPs)	iorea fluorece	nt proteins
	Common			Rel. fl.
Mutation	name	$\Box_{\rm exc}(\mathbf{\epsilon})$	$\Box_{cm}(QY)$	@ 37°C
Class 1, wild-type	name	exc(c)	cm(Q1)	<u> </u>
None or Q80R	Wild type	395-397 (25-30)	504 (0.79)	6
None of Quok	w nu type	470-475 (9.5-14)	304 (0.73)	U
F99S, M153T, V163A	Cycle 3	397 (30)	506 (0.79)	100
1 993, W1331, V 103A	Cycle 3	475 (6.5-8.5)	300 (0.79)	100
Class 2, phenolate anion		473 (0.3-6.3)		
S65T		489 (52-58)	509-511 (0.64)	12
F64L, S65T	EGFP	488 (55-57)	507-509 (0.60)	20
F64L, S65T, V163A	LGIT	488 (42)	511 (0.58)	54
S65T, S72A, N149K,	Emerald	487 (57.5)	509 (0.68)	100
M153T, I167T	Emeralu	467 (37.3)	.309 (0.08)	100
Class 3, neutral phenol				-
S202F, T203I	Н9	200 (20)	511 (0 60)	12
	H9-40	399 (20)	511 (0.60)	13
T203I, S72A, Y145F		399 (29)	511 (0.64)	100
	kea π-electron system	(yellow fluorescent proteins) (YFPs)	1 500 (0 50)	
S65G, S72A, T203F	EMED	512 (65.5)	522 (0.70)	6
S65G, S72A, T203Y	EYFP	508 (48.5)	518 (0.78)	12
S65G, V68L, Q69K	10C Q69K	516 (62)	529 (0.71)	50
S72A, T203Y	100	614 (02.4)	505 (0 (1)	***
S65G, V58L, S72A, T203Y	10C	514 (83.4)	527 (0.61)	58
S65G, S72A, K79R,	Topaz	514 (94.5)	527 (0.60)	100
T203Y	D			
F46L	EYFP-F46L	515 (78.7)	528 (0.61)	ND
F64L, M153T, V163A, S175G	SEYFP	515 (101)	528 (0.56)	ND
F46L, F64L, M153T, V163A,				
S175G	SEYFP-F46L	see: Nagai et al., Nature Biotech. 20:		
	('Venus')	87-90, 2002		
V68L, Q69M	( ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' ' '	0. 50, 2002		
. 002, 20,111	'Citrine'	see: Griesbeck et al., J. Biol. Chem		
		276: 29188-29194 (2001)		
V163A, T203H		270.25100 25151 (2001)		
	PA-GFP	see: Patterson et al., Science 297:		
•		1873-1877, 2002		
Class 5, indole in chromophore (cy	an fluorescent proteir			
Y66W	1	436	485	
Y66W, N146I, M153T,	W7	434 (23.9)	476 (0.42)	61
V163A	'''	452	505	01
F64L, S65T, Y66W,	W1B or	434 (32.5)	476 (0.4)	80
N146I, M153T, V163A	ECFP	452	505	50
S65A, Y66W, S72A,	WIC	435 (21.2)	495 (0.39)	100
N146I, M153T, V163A	•	135 (21.2)	155 (0.55)	100
T203Y	CGFP	see: Sawano & Miyawaki, Nucleic		
	30	Acid Res. 28: E78 (2000)		
Class 6, imidazole in chromophore	(blue fluorescent pro		1	
Ү66H	BFP	384 (21)	448 (0.24)	18
Y66H, Y145F	P4-3	382 (22.3)	446 (0.24)	52
F64L, Y66H, Y145F	EBFP	380-383 (26.3-31)	440 (0.3)	100
		350-363 (20.3-31)	(0.17-0.26)	100
Class 7, phenyl in chromophore	<u> </u>		(0.17-0.20)	
Y66F	I	260	1 442	
TOOF	L	360	442	

5	fluorescent (GFP) (SEQ I wavelength mammalian c No:3),(cyan recent vari	lignment of wild type Aequorea victoria GFP and Aequorea-derived proteins (Zhang et al. 2002). New variants of green fluorescent protein D No:2) that encode proteins with altered excitation and emission properties relative to wild type GFP are aligned. These include the codon-usage optimized ECFP (cyan) (SEQ ID No:8), EGFP (green) (SEQ ID No:1), EGFP (green), and EYFP (yellow) (SEQ ID No:4) variants. Three more ants of EYFP include EYFP-Q69M (Citrine) (SEQ ID No:5), super-EYFP (SEYFP) 6), and SEYFP-F46L ('Venus') (SEQ ID No:7).
15	GFP EGFP EYFP EYFP-Q69M	1 -MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPT 1 MV
	SEYFP SEYFP-F46L ECFP	1 MVLLL
20	GFP EGFP EYFP EYFP-Q69M	60 LVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTL 61LT
25	SEYFP SEYFP-F46L ECFP	61LGLA
30	GFP EGFP EYFP-Q69M SEYFP SEYFP-F46L ECFP	120 VNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLA         121         121         121         121         121         121         121         121         121         121         1 <tr< td=""></tr<>
40	GFP EGFP EYFP EYFP-Q69M SEYFP SEYFP-F46L ECFP	180 DHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK         181

Fluorescent proteins from species other than Aequorea victoria have also been isolated and characterized. The growing list includes a green fluorescent protein from Renilla reniformis, and a number of fluorescent proteins from the coral Anthozoa. These include the red fluorescent protein from Discosoma (DsRed) (M.V. Matz et al., 1999, Nature Biotech. 17:969-973) which has been crystallized (Yarbrough et al., 2001, Proc. Natl. Acad. Sci. 98: 462-467) and has found wide applicability as a biology tool. Although the coral fluorescent proteins have only 26-30% sequence identity with Aequorea GFP, they are remarkably similar structurally. In particular, the coral fluorescent proteins share the same β-can fold first observed in GFP. All the key secondary

structure elements observed in GFP could be easily detected in the coral proteins in the same arrangements, and remarkable similarity was observed in the stretches forming the "caps' of the can. Key residues thought to be involved in chromophore formation in GFP are also conserved in the coral proteins, including an Arginine at residue 96, the Tyrosine at residue 66 and Glycine at residue 67.

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The structural homology of fluorescent proteins among various species means that many of the principles of genetic engineering and protein engineering previously applied to GFP can also be applied to these fluorescent proteins to create variants with desirable properties for biological applications and biotechnology.

The availability of a bright orange-red fluorescent protein with a high quantum yield would be particularly useful for biological studies as it is spectrally distinct from the previously described green, yellow and cyan variants of GFP. DrFP583, commonly known as DsRed, is a 28-kDa polypeptide that has essentially the same chromophore as GFP, which is autocatalytically formed from an internal Gln-Tyr-Gly (residues 66-68) tripeptide. DsRed is remarkably similar structurally to A. victoria GFP. In fact, the overall fold of DsRed is virtually identical to GFP, consisting of a slightly irregular 11-stranded beta-barrel (described as a beta can) with a coaxial central helix and alpha-helical caps on the barrel ends. The sequence alignment of the coral fluorescent proteins with Aequorea GFP is shown in Table 4.

A number of mutant versions of DsRed have now been described with faster rates of chromophore maturation than the wild-type protein (B.J. Bevis and B.S. Glick, Nature Biotech. 20: 83-86, 2002). Importantly, DsRed has recently been engineered into a monomeric form (mRFP) (R.E. Campbell et al.,June 11, 2002, "A monomeric red fluorescent protein", Proc. Natl. Acad. Sci. 99(12): 7877-7882) which is more useful than the multimeric protein as a reporter.

mRFP1 is a monomer, the signal matures >10-fold faster than for DsRed, and the monomeric protein has minimal emission at wavelengths suitable for excitation of GFP.

A unique GFP-like chromoprotein asCP from the sea anemone Anemonia sulcata was recently discovered (Chudakov,D.M., et al. 2003, Kindling fluorescent proteins for precise in vivo photolabeling". Nat. Biotechnol. 21, 191-194). asCP is initially nonfluorescent, but in response to intense green light irradiation it becomes brightly fluorescent (kindles) with emission at 595 nm. Kindled asCP relaxes back to the initial nonfluorescent state with a half-life of <10 seconds. Alternatively, fluorescence can be "quenched" instantly and completely by a brief irradiation with blue light. A mutant (asCP A148G, or KFP1) has been generated which is capable of unique irreversible photoconversion from the nonfluorescent to a stable bright-red fluorescent form that has 30 times greater fluorescent intensity than the unkindled protein. This "kindling fluorescent protein" can be used for precise in vivo photolabeling to track the movement of cells, organelles and proteins.

Fluorescent proteins have proven to be useful reporters for monitoring gene expression and protein localization in vivo and in real time (J.M. Tavare et al., 2001, J. Endocrinol. 170: 297-306; Thastrup et al., US 6,518,021). Such assays measure cellular events linked to individual proteins, as compared with binary or higher-order events. A number of other useful applications of fluorescent proteins have been described, including the construction of biochemical sensors and the creation of innovative fusion constructs to analyze protein dynamics in living cells. For the measurement of bimolecular events, FRET (fluorescence resonance energy transfer) or BRET (bioluminescence resonance energy transfer) assays have been well described (A. Miyawaki & R. Tsien, 2000, Methods in Enzymology 327: 472-500; G.W. Gordon et al., 1998, Biophys. J. 74: 2702-2713). GFP, BFP, CFP and RFP have been used in FRET or

BRET assays to detect protein-protein interactions, monitor protease activity, and create calcium indicators, among other uses.

It is important to note that all the above-mentioned applications rely upon tagging of proteins of interest with a functional, full-length (or substantially full-length) fluorescent protein (lumiphore). None of the references cited above describe compositions or uses of <u>fragments</u> of fluorescent proteins.

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Protein-fragment complementation assays (PCA) represent a general method for the construction of assays for the detection and quantitation of biomolecular and drug interactions (J.N. Pelletier, J.N., Remy, I. and Michnick, S.W. 1998, Protein-Fragment complementation Assays: a general strategy for the in vivo detection of Protein-Protein Interactions, J. Biomolecular Techniques 10:32-19; Remy, I., Pelletier, J.N., Galarneau, A. & Michnick, S.W. 2002, Protein Interactions and Library Screening with Protein Fragment Complementation Strategies, in: Protein-protein Interactions: A Molecular Cloning Manual, Cold Spring Harbor Laboratory Press Chapter 25, 449-475; Michnick, S.W., Remy, I., C.-Valois, F.X., Vallee-Belisle, A., Galarneau, A. & Pelletier, J.N., 2000, Detection of Protein-Protein Interactions by Protein Fragment Complementation Strategies, Parts A and B, in: Methods in Enzymology 328:208-230.; J. N. Pelletier & S. W. Michnick. ,1997, A Strategy for Detecting Protein-Protein Interactions in vivo Based on Protein Fragment Complementation. Protein Engineering, 10(Suppl.): 89.).

PCA involves the oligomerization-assisted complementation of fragments of a reporter protein such as a monomeric enzyme, a fluorescent protein, luminescent protein or phosphorescent protein. Dimeric and multimeric enzymes can also be used in PCA, however, monomeric proteins are preferred. As described by Michnick et al. (US 6,270,964) the ideal

properties of a protein suitable for PCA are: 1) a protein or enzyme that is relatively small and monomeric; 2) for which there is a large literature of structural and functional information; 3) for which simple assays exist for the reconstitution of the protein or activity of the enzyme; and 4) for which overexpression in eukaryotic and prokaryotic cells has been demonstrated.

Figure 1 of US 6,270,964 shows a general description of a PCA. The gene for a protein or enzyme is rationally dissected into two or more fragments. Using molecular biology techniques, the chosen fragments are subcloned, and to the 5' ends of each, proteins that either are known or thought to interact are fused. Co-transfection or transformation of these DNA constructs into cells is then carried out. Reassembly of the probe protein or enzyme from its fragments is catalyzed by the binding of the test proteins to each other, and reconstitution is observed with some assay. It is crucial to understand that these assays will only work if the fused, interacting proteins catalyze the reassembly of the protein or enzyme. That is, observation of reconstituted protein or enzyme activity must be a measure of the interaction of the fused proteins.

US 6,270,964 taught the principles, methods and applications of PCAs for a large number of useful reporters that can generate a fluorescent signal (see Table 1). Example 3 of that patent describes various embodiments of PCAs including a number of specific reporters suitable for PCA. Details were described for glutathione-S-transferase, firefly luciferase, xanthine-guanine phosphoribosyl transferase (XPRT), diaphorase, adenosine deaminase, bleomycin binding protein, hygromycin-B-phosphotransferase, histidinol NAD+oxidoreductase and Aequorea green fluorescent protein (GFP). Table 1 of US 6,270,964 described an even larger list of other reporters meeting the criteria for PCA.

In Example 3 of US 6,270,964 a PCA based on GFP was described including its properties and advantages: "GFP from Aequorea victoria is becoming one of the most popular protein markers for gene expression. This is because the small, monomeric 238 amino acids protein is intrinsically fluorescent due to the presence of an internal chromophore that results from the autocatalytic cyclization of the polypeptide backbone between residues Ser65 and Gly67 and oxidation of the bond of Tyr 66. The GFP chromophore absorbs light optimally at 395 nm and possesses also a second absorption maximum at 470 nm. This bi-specific absorption suggests the existence of two low energy conformers of the chromophore whose relative population depends on the local environment of the chromophore. A mutant Ser65Thr that eliminates isomerization results in a 4 to 6 times more intense fluorescence than the wild type. Recently the structure of GFP has been solved by two groups, making it a candidate for a structure-based PCA design which we have begun to develop. As with the GST assay we are doing all of our initial development in E. Coli with GCN4 leucine zipper-forming sequences as oligomerization domains. Direct detection of fluorescence by visual observation under broad spectrum UV light will be used. We will also test this system in COS cells, selecting for cotransfectants using fluorescence activated cell sorting." The issued claims of US 6,270,964, US 6,294,330 and US 6,428,951 include fluorescent proteins in addition to other reporter classes. PCAs have been used to screen diverse peptide libraries (J.N. Pelletier, et al., 2000, Nature Biotech. 17: 683-690) and cDNA or antibody libraries (E. Moessner et al., 2001, J. Mol. Biol. 308: 115-122; I. Remy et al., submitted for publication); to quantify the association constants of protein domains such as parallel and anti-parallel leucine zipper-forming sequences (K.M. Arndt et al., 2000, J. Mol. Biol. 295: 627-639; I. Ghosh et al., 2000, J. Am. Chem. Soc 122:5658-5659); to detect the drug-induced association and dissociation of protein complexes (I. Remy and

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S.W. Michnick, 1999, Proc Natl Acad Sci USA 96: 5394-5399); to measure the ligand-induced activation of cellular receptors (I. Remy et al., 1999, Science 283: 990-993); to study transcription factor complexes in live cells (R. Subramaniam et al., 2001, Nature Biotech. 19: 769-772, 2001); to quantitate elements of signal transduction pathways in real time (I. Remy and S.W. Michnick, 2001, Proc Natl Acad Sci USA,98: 7678-7683, 2001; A. Galarneau et al., 2002, Nature Biotech. 20: 619-622); and to pinpoint the subcellular locations of protein-protein complexes (I. Remy and S.W. Michnick, 2001, Proc Natl Acad Sci USA 98: 7678-7683; R. Subramaniam et al., 2001, Nature Biotech., 19: 769-772; C.-D. Hu et al., Molecular Cell 9: 789-798, 2002; H. Yu et al., submitted for publication).

Subsequent to our inventions describing the use of GFP for PCA, Ghosh et al. (J. Am. Chem. Soc 122:5658-5659, 2000; US 2002/0146701) used a GFP PCA to study GCN4 leucine zipper oligomerization in a manner originally proposed by Michnick et al. They showed antiparallel leucine zipper-directed reassembly of GFP fragments in bacteria. A single GFP variant was chosen for these studies and a single fragmentation site was used. The authors did not disclose additional principles or methods for fragmenting a fluorescent protein based on rational design beyond the principles first described in Michnick et al. (e.g. US 6,270,964). Moreover, other than the fragment pair used in the leucine zipper study, Ghosh and coworkers did not disclose specific assay compositions useful for PCA.

Hu et al. (Molecular Cell 9: 789-798, 2002) described a PCA based on a yellow variant of GFP, where the fragments of YFP were fused either to parallel leucine zippers or to Rel family proteins. However, additional principles and methods of engineering fluorescent proteins, and fragment compositions, were not described by Hu and coworkers. Moreover, the prior art is silent on the topic of whether mutations known to affect the properties of intact fluorescent

proteins would confer similar properties on polypeptide fragments used for PCA.

Since fluorescent protein PCAs do not depend upon external cofactors or substrates for signal generation, they are particularly useful for the construction of cell-based assays. A suite of fluorescent protein PCAs would enable a large number of useful assays with differing spectral properties. For example, fluorescent proteins with high quantum yields could be engineered into PCA fragments to allow detection of rare events within cells, such as complexes between proteins expressed at very low levels, or low-affinity complexes between enzymes and their substrates. In addition, PCAs with red-shifted emissions would provide improved signal to noise relative to cellular autofluorescence which often occurs in the green channel. Importantly, fragments generating different color PCAs could be combined to allow simultaneous monitoring of two, three, or more cellular events (multicolor PCA). Finally, fluorescent protein PCAs could be used to create multicolor arrays for rapid diagnostics. For example, multicolor arrays based on antibodies binding to different antigens would allow the rapid and simultaneous detection of bio-warfare agents.

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#### OBJECTS AND ADVANTAGES OF THE INVENTION

It is an object of the present invention to provide methods for designing and engineering fluorescent protein fragments and mutant fragments for PCA.

It is a further object of the invention to describe a large number of fragment pairs and compositions useful for PCA.

Another object of the invention is to teach that any useful sequence variant of an intact fluorescent protein can be engineered into the PCA fragments, generating assays with a variety of spectral and physical properties.

A further object of the invention is to provide compositions of PCA fragments, incorporating a wide range of mutations that confer useful properties.

A still further object of the invention is to provide multicolor PCAs'.

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The advantage of the invention is the ability to create 'designer' PCAs with a range of useful properties for a variety of applications.

#### SUMMARY OF THE INVENTION

The present invention relates to a composition comprising complementary fragments of a protein, said fragments generating an optically detectable signal when associated.

The invention also relates to fragments derived form fluorescent proteins and mutant fluorescent proteins.

The instant invention also describes complementary fragments of mutant fluorescent proteins which differ from the corresponding fragments of the wild-type protein by at least one amino acid.

The invention further relates to complementary fragments selected from the group consisting of: Seq. ID NO: 20 to Seq. ID NO: 1067.

The invention also describes a composition selected from Seq. ID NO: 20 to Seq. ID NO: 1067 which are further fused to a separate molecule.

The invention also provides a composition comprising complementary fragments of a mutant protein, said fragments generating an optically detectable signal when associated, wherein each fragment is fused to a separate molecule.

The invention is further directed to protein fragment complementation assays for the detection of molecular interactions comprising a reassembly of separate fragments from an

optically detectable protein wherein reassembly of the fragments is operated by the interaction of molecular domains fused to each fragment, wherein reassembly of the fragments is independent of other molecular processes and wherein said reassembly is detected by means of reconstitution of activity of said optically detectable protein.

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The invention also provides a method for detecting biomolecular interactions said method comprising: (a) selecting an appropriate optically detectable protein; (b) effecting fragmentation of said optically detectable protein such that said fragmentation results in reversible loss of protein function; (c) fusing or attaching fragments of said optically detectable protein separately to other molecules; (d) reassociating said protein fragments through interactions of the molecules that are fused or attached to said fragments; and (e) detecting the resulting optical signal.

The present invention also concerns the design and engineering of protein-fragment complementation assays based on fluorescent proteins. Methods for fragmenting fluorescent proteins and creating mutant fragments with specific properties are described, based on fluorescent proteins derived from Aequorea, Anthozoa and Anemonia species. Finally, a large number of fragment compositions and fragment pairs are provided that incorporate mutations with useful properties generating green, yellow, cyan, blue or red signals. Detailed examples of fluorescent protein PCAs are shown with numerous mutants of Aequorea fluorescent proteins, demonstrating the engineering principles and showing that mutations conferring useful properties to the full-length protein can also be conferred to the fragments. The invention also provides methods and compositions for the construction of multi-color PCAs.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1a and 1b show regions in which Aequorea fluorescent proteins can be

fragmented. Figure 1A shows six alternative loops (asterisked) where fragmentation can be effected relative to the linear sequence. Figure 1B shows specific amino acid residues at the sites (see arrows), relative to the three-dimensional structure of the protein; amino acid residues at the fragmentation sites are numbered relative to wild-type GFP.

Figure 2 depicts the strategy for design and creation of a fluorescent protein-fragment complementation assay.

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Figure 3 shows photomicrographs of PCA results in live cells, depicting the relative fluorescent intensities achieved with PCAs based on two variants of A. victoria (GFP PCA and YFP PCA); several different protein-protein complexes were evaluated by fluorescence microscopy 24 hours after transient transfection.

Figure 4 shows PCAs based on fragment pairs generated from several alternative fragmentation sites as depicted in Fig. 1a and 1b.

Figure 5 shows that specific mutations enhance the fluorescent intensities of reassembled fragments, comparing two sequence variants of a YFP PCA that differ by two amino acids; the proteins fused to the complementary fragments are the protein kinases MEK and ERK.

Figure 6 shows the bright signal generated by a super-enhanced PCA (panel a) with mutations that enhance the folding of YFP fragments as compared with a non-enhanced PCA (panel b). The sub-cellular location of protein-protein complexes can also be seen by fluorescence microscopy. Individual YFP fragments are incapable of fluorescing (c and d).

Figures. 7a and 7b shows the effect of engineering additional mutations into fragments in order to enhance the fluorescent intensities of the final PCA, creating an intense fluorescent PCA

(IFP PCA) and allowing for the detection of protein-protein interactions with low (nanogram to sub-nanogram) quantities of DNA.

Figure 8 shows that PCA based on mutant fragments of fluorescent proteins can be used in high-content assays, for example, to detect a change in subcellular localization of protein-protein complexes upon stimulation of living cells by a cytokine as shown here for p65/p50.

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Figure 9 demonstrates a spectrally shifted PCA based on mutant fluorescent protein fragments generating a blue signal in the presence of a protein-protein interaction

Figure 10 demonstrates multi-color PCA, wherein a single fusion protein (p65 in this example) tagged with a fragment corresponding to the C-terminus of a fluorescent protein is capable of generating two different fluorescent PCAs in the same cell depending upon the amino acid sequence of the reporter fragment fused to the proteins that interact with the first protein. Multi-color PCAs allow for the detection and quantification of different protein-protein complexes within the same cells.

### DETAILED DESCRIPTION OF THE INVENTION

Fluorescent proteins are particularly attractive for PCAs because they require no external substrates or probes for the generation of the fluorescent signal. However, fluorescent proteins present certain design challenges because of their unique structure and the requirement for internal formation of an active chromophore for generation of the fluorescent signal. The present invention encompasses the design criteria for fragmentation of a fluorescent protein, which are described below.

Figure 2 describes the steps involved in creating a PCA based on a fluorescent protein.

The first step is the selection of a fluorescent protein and its corresponding DNA sequence. Any

fluorescent protein can be used for PCA based on the design principles that are the subject of the present invention. The choice of a fluorescent protein depends on the desired wavelength, instrumentation, and sensitivity required for the assay of interest. DNA fragments of the selected reporter are then made, using one of the methods described herein. In the first example, we describe the use of rational design to determine where to fragment a fluorescent protein. Because the fluorescent proteins have similar structures, we first describe the design principles for the example of A. victoria GFP.

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GFP is an 11-stranded  $\beta$ -barrel with the highly unusual feature of having an  $\alpha$ -helix that is thread through the central axis of the β-barrel (Ormo et al., Science 273:1392–95, 1996; F. Yang et al., Nat. Biotechnol. 14:1246-5121, 1996; R. Heim et al., Nature 373: 663-64, 1995). Fig. 1A shows a two-dimensional view of the protein; the 11 strands of the barrel are shown in relation to the central alpha-helix, and the amino acid positions at the ends of the barrel are numbered. Fig. 1B shows the 3-dimensional structure of the folded protein; the positions of specific residues at the loops are noted (all numbering is relative to the wild-type GFP). The chromophore is attached to the  $\alpha$ -helix and is buried almost completely in the center of the  $\beta$ barrel cylinder. Almost all the primary sequence is used to build the β-barrel and axial helix. The chromophore is a p-hydroxybenzylidene-imidazolinone formed from residues 65-67, which are Ser(Thr)-Tyr-Gly in the native protein The chromophore (1,2). hydroxybenzylidene)imidazolidin-5-one attached to the peptide backbone through the 1- and 2positions of the ring. First, GFP folds into a nearly native conformation, then the imidazolinone is formed by nucleophilic attack of the amide of Gly67 on the carbonyl of residue 65, followed by dehydration. Finally, molecular oxygen dehydrogenates the Cα-Cβ bond of residue 66 to put its aromatic group into conjugation with the imidazolinone (3).

There are obvious features of the structure that should not be disrupted and therefore, by default, alternatives to such regions are chosen for fragmentation. The design criteria for fluorescent protein PCAs include the following:

(1) Fragmentation is made in  $\beta$ -turns or loops at the extreme ends of individual strands so as not to disrupt the barrel structure. Preferred regions for fragmentation are shown in Fig. 1A, with specific amino acids corresponding to the regions shown in the 3-dimensional structure in Fig. 1B.

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(2) The chromophore is deeply buried in the  $\beta$ -barrel. It is likely that this is required to isolate the chromophore coding sequence, both to assure efficient formation of the chromophore and to maintain both rigid conformation and coordination to the side chains of other amino acids that provide GFP with its unique spectral characteristics. Isolation of the chromophore from solvent is maintained both by being embedded in the  $\beta$ -barrel and also by barrel "capping" structures at either end of the barrel. These caps include: (a) Cap at N-terminus of internal helix: Residues 19-30 strand; Residues 133-143 hairpin; Residues 50-57 (This is the N-terminus of the internal helix); and (b) Cap at C-terminus of internal helix: Residues 1-10, helix; Residues 77-98, helix; Residues 191-197, strand. Neither of these regions should be disrupted.

Based on these criteria the optimal fragmentation regions are shown in Figure 1a relative to the linear model of the GFP structure. Fragmentation can be effected in one of the loops comprising amino acid residues 38-40 (region 1); amino acids 101-103 (region 2); amino acids 114-118 (region 3); amino acids154-160 (region 4); amino acids 171-175 (region 5); or amino acids 188-190 (region 6). Fragmentation can be effected at one of the amino acids within those regions. It will be obvious to one skilled in the art that the exact residue at which the fragmentation is effected may vary within the designated loops without having a significant

impact on the ability of the fragments to fold and reconstitute an active structure, as long as the design criteria described above and in US 6,270,964 are followed. To prove the design principle, we present examples (Figure 4) of successful PCA construction based on fragmentation of YFP at three different amino acids selected from the regions listed above.

While fragmentation of proteins for PCA is generally based on rational dissection of the polypeptide chain as described in the present invention, a number of other engineering approaches can be used that will be well known to one skilled in the art. For example, we have previously proposed an alternative approach based on the use of 5' exonucleases to generate libraries of fragments to search for optimal pairs (Michnick, et al. 6,270,964).

In the present invention we generated fragments of the full-length cDNA for GFP using PCR to amplify fragments of interest. Alternatively, oligonucleotides encoding fragments can simply be synthesized using standard oligonucleotide synthesis techniques; this approach was taken to generate a PCA based on a cyan fluorescent protein (Figure 9). In a preferred embodiment, mutant fragments of a fluorescent protein are used, having properties tailored to the biological application and the instrumentation to be used. To generate mutant fragments, as described below in detail, we utilized site-directed mutagenesis of GFP in order to obtain fragments that when reconstituted would have altered fluorescence properties or superior folding or maturation rates and stabilities. Site-directed mutagenesis is achieved by any of a number of approaches that are well known to one skilled in the art (see MM Ling & BH Robinson, 1997, Approaches to DNA mutagenesis: an overview. Anal Biochem 254:157-78). Selected examples of such methods are provided here; however, these examples are not intended to be limiting for the practice of this invention. Suitable methods could include combinations of random mutagenesis and directed evolution or DNA shuffling schemes (A.L. Kurtzman et al., 2001,

Advances in directed protein evolution by recursive genetic recombination: applications to therapeutic proteins, Curr Opin Biotechnol 2001 Aug;12(4):361-70; SW Santoro et al., 2002, Directed evolution of the site specificity of Cre recombinase. Proc Natl Acad Sci U S A 2002 99:4185-90; Z. Shao et al., 1996, Engineering new functions and altering existing functions, Curr Opin Struct Biol 6:513-8; S. Harayama, 1998, Artificial evolution by DNA shuffling, Trends Biotechnol 1998, 16:76-82); assembly PCR or gene synthesis approaches (WP Stemmer Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides, Gene 164(1):49-53; RM Horton et al. 1993, Gene splicing by overlap extension. Methods Enzymol. 217:270-9), or fragmentation by exo- or endo-nuclease digestion (M. Kitabatake and H. Inokuchi, 1993, A simplified method for generating step-wise deletions using PCR, Gene 123:59-61; S. Henikoff, 1990, Ordered deletions for DNA sequencing and in vitro mutagenesis by polymerase extension and exonuclease III gapping of circular templates, Nucleic Acids Res 18(10):2961-6). A particularly powerful method is based on 5'-template-assisted long-range plasmid polymerization as exemplified by a number of commercial mutagenesis kits, for example the QuickChange<sup>TM</sup> system (Stratagene). In addition, various forms of directed evolution based on DNA shuffling could also be used to generate completely novel PCAs.

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Once the DNA fragments F1 and F2 of the gene encoding the fluorescent protein are generated, each fragment is individually fused in frame with a gene encoding a protein or polypeptide of interest in a suitable expression vector. A variety of standard or novel expression vectors can be chosen based on the cell type and desired expression level; such vectors and their characteristics will be well known to one skilled in the art. Optimally, a flexible linker, such as that described in Example 1 below, is fused between the fluorescent protein fragment and the

gene of interest to facilitate fragment complementation. Therefore, each expression vector codes for a fusion protein consisting of an operably linked gene of interest, a flexible linker, and either F1 or F2 of the chosen fluorescent protein. As shown in Figure 2, since either F1 or F2 can be fused to the gene of interest and the orientation of the fusion can be either 5' or 3' relative to the gene of interest, four different DNA constructs are possible for any single gene of interest. (It should be noted that if the fluorescent protein fragment is at the 5' end of the construct, it will be preceded by an initiating methionine (atg codon), whereas if the fragment is at the 3' end of the construct, the gene of interest will be preceded by the initiating methionine (atg codon)). Therefore, the invention covers not only F1 fragments that have a naturally occurring initiating methionine, but also the same F1 fragments that have been modified to remove the initiating methionine when the F1 fragment is to be at the 3' end of the construct. Similarly, the invention covers F2 fragments that naturally do not begin with an initiating methionine, but also those same F2 fragments that have been modified include an initiating methionine when the F2 fragment is to be at the 5' end of the construct.

To generate the PCA for a pair of proteins A and B, constructs encoding A and B fused separately to complementary fluorescent protein fragments F1 and F2 are co-transfected into cells. If proteins A and B interact, fragments F1 and F2 are brought into close proximity where they are capable of folding and reconstituting an active chromophore. The fluorescent signal can then be measured by a variety of standard methods, including fluorescence spectroscopy, flow cytometry (FACS), or microscopy. All of these methods can be used in automated, high-throughput formats using instrumentation well known to those skilled in the art. As described below, novel multicolor fluorescent PCAs can also be generated by using more than two construct pairs simultaneously. Finally, although it is expedient to carry out the engineering and

construction of PCAs at the DNA level and then either allow a cell to produce the fusion proteins, it is not essential. For example, fusion proteins can be made in vitro using in vitro expression techniques that are well known to those skilled in the art. In addition, for in vitro PCAs, fusion polypeptides could be produced synthetically by peptide synthesis, or by ligation of peptide fragments encoding molecules of interest to create peptide fusions with the fluorescent protein fragments.

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The structural homology amongst fluorescent proteins from various marine organisms enables the same design criteria described for GFP to be applied to other fluorescent proteins such as those recently described from Discosoma and from Anemonia. Therefore, in addition to GFP variants we present additional PCAs based on fragments of the monomerized Red Fluorescent Protein (mRFP1, derived from DsRed); and a fluorescent protein KFP1 that can be transiently activated (kindled) by irradiation of the chromophore at specific wavelengths based on the fluorescent protein 'asCP' from *Anemonia sulcata*. (Chudakov, D. M., Belousov, et al. 2003, Nat Biotechnol 21, 191-194). In both cases the fluorescent proteins are homologues of GFP at the amino acid level.

The red-shifted fluorescent PCAs that are the subject of this invention will be particularly useful for biological applications in which there is significant auto-fluorescence in the green channel. For example, red-shifted PCAs will be particularly useful for cDNA library screening applications using flow sorting. in this case the positive cell population expressing a protein-protein complex detected by an RFP PCA will be shifted away from the background population, readily allowing flow sorting of the positive cells.

DsRed from Discosoma has been demonstrated to be a structural homologue of GFP. DsRed is a 28-kDa polypeptide that has essentially the same chromophore as GFP, which is

auto-catalytically formed from an internal Gln-Tyr-Gly (residues 66-68) tripeptide. DsRed is remarkably similar structurally to A. victoria GFP. In fact, the overall fold of DsRed is virtually identical to GFP, consisting of a slightly irregular 11-stranded beta-barrel (described as a beta can) with a coaxial central helix and alpha-helical caps on the barrel ends. The novel fragments that are the subject of this invention are based directly on examining of the RFP structure (Wall, M. A., et al., The structural basis for red fluorescence in the tetrameric GFP homolog DsRed, Nat Struct Biol 7, 1133-1138 (2000)) and using the rational design criteria described above for fragmentation of GFP. The amino acid sequence of mRFP1 is shown in Table 5 aligned with the sequence of A. Victoria GFP, showing alignment of the alternative fragmentation sites. The present invention encompasses nucleic acid sequences and polypeptide fragments generated by fragmentation of mRFP1 at the following alternative fragmentation sites: amino acids 38-40 (region 1); amino acids 100-102 (region 2); amino acids 113-117 (region 3); amino acids 152-156 (region 4); amino acids 167-171 (region 5); amino acids 182-184 (region 6). The positions of specific amino acid residues are shown for Glutamate 39 (E39), Aspartate 101 (D101), Aspartate 115 (D115), Glutamate 153 (E153), Aspartate 169 (D169), or Lysine 184 (K184). The fragmentation sites relative to the nucleic acid sequence encoding the full-length mRFP1 polypeptide are depicted in Table 6.

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Table 4. Multiple alignment of coral (Anthozoa sp.) fluorescent proteins. The numbering is based on A. victoria GFP (M.V. Matz et al., Nature Biotech. 17: 969-973, 1999). Two proteins from Zoanthus and two from Discosoma are compared pairwise. Introduced gaps are represented by dots. In the consensus sequences ("cns"), "O" marks an aromatic residue (Phe, Tyr, Trp, His); "@"=bulky hydrophobic residues (Val, Leu, Ile, Met, Phe, Trp); "+"=positively charged residues (His, Arg, Lys); "-"=negatively charged residues (Asp, Glu). "FP', fluorescent protein; 'z', Zoanthus; 'ds', Discosoma; 'dr', Discosoma 'red'; 'c', Clavularia; 'cns', consensus. DrFP583 is commonly referred to as 'DsRed' where 583 refers to the emission maximum at 583 nm (at an excitation maximum of 558 nm). By homology to the fragmentation sites chosen for GFP (Fig. 1A and 1B), alternative fragmentation sites of the coral fluorescent proteins which are the subject of the current invention are underlined.

<sup>10 20 30 40 50</sup> 35 MSKGEELFTG.VVPILVELDGDVNGHKFSVSGEGEGDA<u>TYG</u>KLTLKFICTT.GKLPVP..W GFP (SEQ ID NO:2)

	$\label{eq:magskhgltk.fmtmkyrmegcvdghkfvitgegigyp\underline{F}\underline{K}\underline{G}\underline{K}\underline{Q}\underline{A}\underline{I}\underline{N}\underline{L}\underline{C}\underline{V}\underline{V}\underline{E}\underline{G}\underline{G}\underline{P}\underline{F}\underline{F}\underline{A}\underline{E}\\ \underline{M}\underline{A}\underline{H}\underline{S}\underline{K}\underline{H}\underline{G}\underline{K}\underline{G}\underline{I}\underline{N}\underline{L}\underline{C}\underline{V}\underline{I}\underline{E}\underline{G}\underline{G}\underline{P}\underline{F}\underline{F}\underline{S}\underline{E}\\ \underline{M}\underline{M}\underline{G}\underline{G}\underline{M}\underline{G}\underline{M}\underline{G}\underline{G}\underline{M}\underline{G}\underline{M}\underline{G}\underline{G}\underline{M}\underline{G}\underline{G}\underline{G}\underline{G}\underline{M}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}\underline{G}G$	zFP506 (SEQ ID NO:9) zFP538 (SEQ ID NO:10)				
5	$\label{eq:mscsksvike.emlid} {\tt MSCSKSVIKE.EMLIDLHLEGTFNGHYFFIKGKGKGQPNEGTNTVTLEVT} KGGPLPFGW\\ {\tt MRSSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGRP}\underline{{\tt MSCSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGRP}\underline{{\tt MSCSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGERP}\underline{{\tt MSCSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGERP}\underline{{\tt MSCSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGEGRP}\underline{{\tt MSCSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGEGRP}\underline{{\tt MSCSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGEGRP}{\tt MSCSKNVIKE.FMRFKVRMEGTVNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGHEFFIEGETNGTNGTNGHEFFIEGETNGTNGTNGTNGTNGTNGTNGTNGTNGTNGTNGTNGTNGT$	dsFP483(SEQ ID NO:11) drFP583(SEQ ID NO:12)				
40	MALSNKFIGD.DMKMTYHMDGCVNGHYFTVKGEGNGKP <u>YEG</u> TQTSTFKVTMANGGPLAFSF KALTTMGVIKPDMKIKLKMEGNVNGHAFVIEGEGEGKP <u>YDG</u> THTLNLEVKMAEGAPLPFSY MSKGEELFTG.VVPILVELDGDVNGHKFSVSGEGEGDA <u>TYG</u> KLTLKFICTT.GKLPVPW	amFP486 (SEQ ID NO:13) cFP484 (SEQ ID NO:14) GFP				
10	MAQSKHGLTK, FMTMKYRMEGCVDGHKFVITGEGIGYP <u>FKG</u> KQAINLCVVEGGPLPFAE MAHSKHGLKE.EMTMKYHMEGCVNGHKFVITGEGIGYP <u>FKG</u> KQTINLCVIEGGPLPFSE	zFP506 zFP538				
15	MSCSKSVIKE.EMLIDLHLEGTFNGHYFFIKGKGKGQP <u>NEG</u> TNTVTLEVT KGGPLPFGW MRSSKNVIKE.FMRFKVRMEGTVNGHEFFIEGEGEGRP <u>YEG</u> HNTVKLKVT.KGGPLPFAW	dsFP483 drFP583				
	${\tt MALSNKFIGD.DMKMTYHMDGCVNGHYFTVKGEGNGKP} \underline{{\tt MEG}}{\tt TQTSTFKVTMANGGPLAFSF}\\ {\tt KALTTMGVIKPDMKIKLKMEGNVNGHAFVIEGEGEGKP}\underline{{\tt MG}}{\tt THTLNLEVKMAEGAPLPFSY}\\$	amFP486 cFP484				
20	s M@ EG vnGH F@ GeG G P <u>o G</u> t@ @ V GgPLpF @ @ EG vnGH F @ GeG G <u>G</u> t@ @ P@p	cns. Anthozoa cns. all				
25	60 70 80 90 100 110 PTLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFF <u>KDI</u> GNYKTRAEVK <u>FEGD</u>	GFP				
	DILSAAFNYGNRVFTEYPQDIVDYFKNSCPAGYTWDRSFLF <u>EDG</u> AVCICNADIT <u>VSVEE</u> N DILSAGFKYGDRIFTEYPQDIVDYFKNSCPAGYTWGRSFLF <u>EDG</u> AVCICNVDIT <u>VSVKE</u> N	zFP506 zFP538				
30	HILCPQFQYGNKAFVHHPDNIHDYLKLSFPEGYTWERSMHF <u>EDG</u> GLCCITNDIS <u>LTGN</u> DILSPQFQYGSKVYVKHPADIPDYKKLSFPEGFKWERVMNF <u>EDG</u> GVVTVTQDSS <u>LQDG</u>	dsFP483 dsFP583				
35	DILSTVFKYGNRCFTAYPTSMPDYFKQAFPDGMSYERTFTY <u>EDG</u> GVATASWEIS <u>LKGN</u> DILSNAFQYGNRALTKYPDDIADYFKQSFPEGYSWERTMTF <u>EDK</u> GIVKVKSDIS <u>MEED</u>	amFP486 cFP484				
	dILs F YGn+ f yP @ DYfK sfPeGo wER @ O <u>EDgo</u> @ Dis <u>@</u> iL F YG f yP @ DyfK PeGo ER @ O <u>D</u> g D@ <u>@</u>	cns. Anthozoa cns. all				
40	120 130 140 150 160 170 TLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIM <u>ADKOKNG</u> IKVNFKIRHN <u>IELGS</u> VQL	GFP				
45	CMYHESKFYGVNFPADGPVM.KKMTDNWEPSCEKII <u>PVPKOGI</u> LKGDVSMYLL <u>LKDGG</u> RLR CIYHKSIFNGMNFPADGPVM.KKMTTNWEASCEKIM <u>PVPKOGI</u> LKGDVSMYLL <u>LKDGG</u> RYR	ZFP506 ZFP538				
.0	CFYYDIKFTGLNFPPNGPVV.QKKTTGWEPSTERLYP <u>RDGV</u> LIGDIHHALT <u>VEGGG</u> HYA CFIYKVKFIGVNFPSDGPVM.QKKTMGWEASTERLYP <u>RDGV</u> LKGEIHKALK <u>LKEGG</u> HYL	dsFP483 drFP583				
50	CFEHKSTFHGVNFPADGPVM.AKKTTGWDPSFEKMTV. <u>CDGI</u> LKGDVTAFLM <u>LQGG</u> NYR SFIYEIRFDGMNFPPNGPVM.QKKTLKWEPSTEIMYV <u>RDGY</u> LVGDISHSLL <u>LEGGC</u> HYR c@ O f G@NFP dGPVm KkT WepS E+@ <u>dG@</u> L+GD@ L <u>l GG</u> +y	amFP486 cFP484 cns. Anthozoa				
	@ @ G@nF dG @@ K o @@+ @ <u>@ G</u>	cns. all				
55	180 190 200 210 220 230 ADHYQQNTP <u>IGD</u> G.PVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK	GFP				
60	CQFDTVYKA <u>KSV</u> PRKMPDWHFIQHKLTREDRSDAKNQKWHLTEHAIASGSALP CQFDTVYKA <u>KSV</u> PSKMPEWHFIQHKLLREDRSDAKNQKWQLTEHAIAFPSALA CDIKTVYRAKKAALKMPGYHYVDTKLVIWNNDKEFM.KVEEHEIAVARHHPFYEPKKDK	zFP506 zFP538 dsFP483				
	VEFKSIYMAKKAPVQLPGYYYVDSKLDITSHNEDYT.IVEQYERTEGRHHLFL  CQFHTSYKTKKPVTMPPNHVVEHRIARTDLDKGGN.SVQLTEHAVAHITSVVPF	drFP583				
<b>6</b> 5	CDFKSIYKAKKVKLPDYHFVDHRIEILNHDKDYN.KVTLYENAVARYSLLPSQA  C @ t@YkaKk pvk@P ho@Dh+@ @ 1 E a@a	cFP484				
	p @P hoe @ 1 E a	cns. all				

Table 5. Amino acid alignment of mRFP1(SEQ ID NO:16) with A. victoria GFP (SEQ ID NO:2) showing alternative fragmentation sites (underlined) that are the subject of the present invention

```
# Aligned sequences: 2
# 1: A. victoria GFP
# 2: mRFP1
# Matrix: EBLOSUM62
 Gap penalty: 12
 Extend penalty: 2
 Length: 240
                  58/240 (24.2%)
 Identity:
# Similarity:
                109/240 (45.4%)
# Gaps:
                  17/240 ( 7.1%)
# Score: 186
              10
                                 30
      MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDA<u>TYG</u>KLTLKFICTT
GFP
      mRFP1
      MASSEDVIKEFMRFKVRMEGSVNGHEFEIEGEGEGRP<u>YEG</u>TQTAKLKVTK
              10
                       20
                                 30
                                          40
               60
                        70
                                  80
                                           90
GFP
      G-KLPVPWPTLVTTFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTIF
        {\tt GGPLPFAWDILSPQFQYGSKAYVKHPADIP--DYLKLSFPEGFKWERVMN}
mRFP1
              60
                       70
                                 80
                                            90
    100
              110
                       120
                                 130
                                          140
GFP
      F\underline{KDD}GNYKTRAEVK\underline{FEGDT}LVNRIELKGIDFKEDGNILGHK-LEYNYNSH
                        .. ...:.: .: .:: .. : . .. ...
      FEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASTE
mRFP1
     100
               110
                        120
                                  130
     150
               160
                        170
                                           190
GFP
      {\tt NVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDN}
              :.
                                       ... .. :: ::
      RMY---PEDGALKGEIKMRLKLKDGGHYDAE--VKTTYMAKKPVQLPGA
mRFP1
     150
                  160
                            170
                                       180
                                                 190
                                  230
               210
                        220
GFP
      HYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK
                   ....:
      YKTDIKLDIT---SHNEDYTIVEQYERA----EGRHSTGA
mRFP1
           200
                       210
                                    220
```

Table 6. mRFP1 full length nucleic acid(SEQ ID NO:15) and amino acid sequence(SEQ ID NO:16). Alternative fragmentation sites that are the subject of the present invention are shown at the following regions (underlined): amino acids 38-40 (region 1); amino acids 100-102 (region 2); amino acids 152-156 (region 3); amino acids 167-171 (region 4); amino acids 182-191 (region 5) . The positions of specific amino acid residues at the fragmentation sites for mRFP1 are shown for Glutamate 39 (E39), Aspartate 101 (D101), Aspartate 115 (D115), Glutamate 153 (E153), Aspartate 169 (D169), or Lysine 184 (K184). atg gcc tcc tcc gag gac gtc atc aag gag ttc atg cgc ttc aag gtg cgc atg gag ggc M A S S E D V I K E F M R F K V R M E G tee gtg aac ggc cac gag tte gag ate gag ggc gag ggc gag ggc ege eee <u>Eac gag ggc</u> S V N G H E F E I E G E G E G R P  $\underline{Y}$  E  $\underline{G}$ acc cag acc gcc aag ctg aag gtg acc aag ggc ggc ccc ctg ccc ttc gcc tgg gac atc T T A K L K V T K G G P L P F A W D I 41 50 55 6020 ctg tcc cct cag ttc cag tac ggc tcc aag gcc tac gtg aag cac ccc gcc gac atc ccc L S P Q F Q Y G S K A Y V K H P A D I P 61 65 70 75 8025 gac tac ttg aag ctg tcc ttc ccc gag ggc ttc aag tgg gag cgc gtg atg aac ttc gag Y L K L S F P E G F K W E R V M N F E 81 90 95 100 30 aag gtg aag ctg cgc ggc acc aac ttc ccc tcc gac ggc ccc gta atg cag aag acc V K L R G T N F P S D G P V M Q K K T 121 125 130 135 14035 atg ggc tgg gag gcc tcc acc gag cgg atg tac  $\underline{occ}$  gag ggc  $\underline{ggc}$   $\underline{g$ qaq 40 atc aag atg agg ctg aag ctg aag cac cqc cgc cac tac gac gcc gag gtc aag acc I K M R L K  $\overline{L}$  K D  $\overline{G}$  G H Y D A E V K T 161 165 D169 170 175 45 tac <u>atg gec aac</u> aag ccc gtg cag ctg ccc ggc gcc tac aag acc gac atc aag ctg gac Y  $\underline{M}$   $\underline{A}$   $\underline{K}$  K P V Q L P G A Y K T D I K L D 181 K184 185 190 195 200 50 atc acc tcc cac aac gag gac tac acc atc gtg gaa cag tac gag cgc gcc gag ggc cgc I S H N E D Y T I V E Q Y E R A E G R 210 215 cac tcc acc ggc gcc 55 H S T G

A PCA based on kindling fluorescent protein (KFP1) is also the subject of the present invention. In the case of KFP1, which is a variant of the fluorescent protein derived from Anemonia sulcata, the alternative fragmentation sites are based on the alignment of KFP1 to GFP as shown in Table 7. Table 8 shows the fragmentation sites relative to the full-length nucleotide and amino acid sequence of KFP1.

Table 7. Amino acid alignment of kindling fluorescent protein (KFP1)(SEQ ID NO:18) with A. victoria GFP(SEQ ID NO:2), showing alternative fragmentation sites(underlined) that are the subject of the present invention

```
Aligned sequences: 2
 1: A. victoria GFP
 2: kindling fluorescent protein (KFP1)
 Matrix: EBLOSUM62
 Gap penalty: 12
 Extend penalty: 2
 Length: 241
 Identity:
               57/241 (23.7%)
 Similarity:
               98/241 (40.7%)
 Gaps:
               12/241 ( 5.0%)
 Score: 145
                                  30
GFP
       MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKF-ICT
            :.: ..:. . ..: :::: :. :.:::.
                                             : .:. .
       MAS---LLTETMPFKTTIEGTVNGHCFKCIGKGEGNPFEGTQEMKIEVIE
KFP1
                  10
                                      30
                                                40
                60
                          70
                                    80
                                               90
GFP
       TGKLPVPWPTLVTTFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTIF
        KFP1
       GGPLPFAFHILSTSCMYGSKTFIKYVSGIP--DYFKQSFPEGFTWERTTT
        50
                  60
                            70
                                        80
                                                   90
               110
                         120
                                   130
                                             140
GFP
       FEDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHN
               .. ....: :: .... : .: :: .. .:.
KFP1
       YEDGGFLTAHQDTSLDGDCLVYKVKILGNNFPADGPVMQNKVGRWEPGTE
         100
                   110
                             120
                                       130
                                                  140
               160
                         170
                                   180
                                               190
GFP
       VYIMADKQKNGIKVNFKIRHN<u>IEDGS</u>VQLADHYQQNTP<u>IG--D</u>GPVLLPD
                                      :... : .
                                . .:
       IVYEVDGVLRGQSLMALKCPGGRHLTCHLHTTYRSKKPASALKMPGFHFE
KFP1
         150
                   160
                             170
                                       180
                                                 190
       200
                 210
                           220
                                     230
GFP
       NHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYK
       .: .. .. ..:
                     :. . .. .. ::
KFP1
       DHRIEIMEEVEKGKCYKQYEAAVGRYCDAAPSKLG----HN
         200
                   210
                             220
                                       230
```

Table 8. <u>Kindling fluorescent protein (KFP1) full length nucleic acid (SEQ ID NO:17) and amino acid sequence (SEQ ID NO:18)</u>. Alternative fragmentation sites that are the subject of the present invention are shown at the underlined regions: residues 35-37 (region 1); residues 97-99 (region 2); residues 110-114 (region 3); residues 150-156 (region 4); residues 167-171 (region 5); residues 184-195 (region 6). The positions of specific amino acid residues at the fragmentation sites for KFP1 are shown for Glutamate 36 (E36), Aspartate 98 (D98), Glycine 112 (G112), Valine 153 (V153), Histidine 169 (H169), or Alanine 186 (A186).

10																				
	atg M 1	gcc A	tcc S	ctg L	ctg L 5	acc T	gag E	acc T	atg M	ccc P 10	ttc F	aag K	acc T	acc T	atc I 15	gag E	ggc G	acc T	gtg V	aac N 20
15	ggc G	cac H	tgc C	ttc F	aag K 25	tgc C	atc I	ggc G	aag K		gag E	ggc G	aac N	ccc P	ttc <u>F</u> 35		<u>.g</u>	acc T	cag Q	gag E 40
20	atg M	aag K	atc I	gag E	gtg V 45	atc I	gag E	ggc G	ggc G	ccc P 50	ctg L	ccc P	ttc F	gcc A	ttc F 55	cac H	atc I	ctg L	tcc S	acc T 60
25	tcc S	tgc C	atg M	tac Y	ggc G 65	tcc S	aag K	acc T	ttc F	atc I 70	aag K		gtg V	tcc S	ggc G 75	atc I	ccc P	gac D	tac Y	ttc F 80
30	aag K	cag Q	tcc S	ttc F	ссс Р 85	gag E	ggc G	ttc F	acc T	tgg W 90	gag E	cgc R	acc T	acc T	acc T 95		gag E	gac D D98	ggc <u>G</u>	ggc G 100
	ttc F	ctg L	acc T	gcc A	сас Н 105	cag Q	gac D	acc T	tcc S		gac D		Ď		ctg L 115		tac Y	_	gtg V	aag K 120
35	atc I	ctg L	ggt G	aac N	aac N 125	ttc F	ccc P		gac D	ggc G 130				cag Q	aac N 135	aag K	gtc V	ggc G	cgc R	tgg W 140
40	gag E	ccc P	gga G	acc T	gag E 145	atc I	gtg V		gag E	gt.g <u>V</u> 150	gac D	ggc G	gtg V <b>V15</b> 3	<u> </u>	ogc <u>R</u> 155	ggc <u>G</u>	cag Q	tcc S	ctg L	atg M 160
45	gcc A	ctg L	aag K	tgc C	ccc P 165	ggc G	ggc G	8		I.				ctg L	сас Н 175	acc T	acc T	tac Y	cgc R	tcc S 180
50	aag K	aag K	CCC P	Α.		A	ctg L			ссс Р 190			cac H	ttc F	gag E 195	gac D	cac H	cgc R	atc I	gag E 200
30	atc I	atg M	gag E	gag E	gtg V 205	gag E	aag K	ggc G	aag K	tgc C 210	tac Y	aag K	cag Q	tac Y	gag E 215	gcc A	gcc A	gtg V	ggc G	cgc R 220
55	tac Y	tgc C	gac D	gcc A	gcc A 225	ccc P	tcc s	aag K	ctg L	ggc G 230	cac H	aac N								

It is a feature of PCA that the reassembled fragments are capable of re-creating the activity of the intact reporter from which the fragments are derived. For example, for a PCA based on dihydrofolate reductase (DHFR), the reassembled fragments are capable of binding

methotrexate in a manner similar to the full-length protein (I. Remy & S.W. Michnick, 1999, Proc Natl Acad Sci USA, 96: 5394-5399); in addition, mutations that affect the properties of the intact DHFR protein confer similar properties to the DHFR fragments when they are used in PCA (J.N. Pelletier, F.-X. C.-Valois & S.W. Michnick, 1998, Proc Natl Acad Sci USA 95: 12141-12146). Similarly, fragments of β-lactamase used in PCA are capable of cleavage of cephalosporin substrates with kinetics similar to the intact β-lactamase protein, and mutations that disrupt the molten globule structure of the intact protein improve the enyzymatic properties of the reassembled fragments (A. Galarneau, M. Primeau, L.-E. Trudeau & S.W. Michnick, 2000, Nature Biotechnol. 20: 619-622).

Since the spectral properties of fluorescent proteins are critically dependent upon the orientation and proximity of amino acids relative to the core chromophore, it is not obvious that mutations that affect the spectral properties of an intact fluorescent protein would have the same effect when engineered into fragments of the protein. We reasoned that, if mutations that affect the spectral properties of fluorescent proteins could be engineered into protein-fragment complementation assays, it would be possible to generate a wide variety of PCAs with various spectral properties. Moreover, the availability of different color PCAs would enable the engineering of designer PCAs for a variety of applications in biology and biotechnology.

To demonstrate this principle, we created PCAs based on numerous variants of A. victoria green fluorescent protein and tested them by creating fusion constructs with several different human genes known to be involved in protein-protein interactions in mammalian cells. In the first example, fragments were generated for PCA by fragmenting an enhanced green fluorescent protein ("EGFP" in Table 3) in order to create a green fluorescent PCA (GFP PCA). The GFP fragments were then further mutated to create novel fragments having the mutations

S65G/V68L/S72A/T203Y which corresponds to the yellow fluorescent protein (YFP) variant named "10C" in Table 2, also referred to as enhanced yellow fluorescent protein ("EYFP") as in Table 3. With intact GFP, the introduction of the S65G/V68L/S72A/T203Y mutations into results in a protein with excitation and emission maxima at 514 nm and 527 nm, respectively, in which the chromophore matures fourfold faster than for the wild type GFP, generating a bright signal for cell biology applications. We sought to determine whether this GFP variant could be used in PCA and to assess the relative intensities of this YFP PCA versus the GFP PCA in cells transiently co-transfected with fragments fused to full-length proteins that had been previously reported to interact in human cells; this analysis is described in detail in Example 1, below.

To demonstrate the utility of the various fragment pairs that are the subject of the invention, we selected three of the fragmentation sites depicted in Fig. 1b and constructed PCAs based on YFP fragments fused to known interacting proteins in various gene/fragment orientations (NN, NC, CN and CC). In Example 2, described below, the results showed that the different fragmentation sites could in fact be used to construct alternative PCAs with good signals vs. background.

In two further examples of the engineering of PCAs based on mutant fragments, we further mutated the YFP fragments in order to determine if mutations shown to enhance the brightness of full-length YFP at physiological temperatures would confer similar properties when engineered into fragments for PCA. First, we engineered two additional mutations, S64L and M153T into YFP[1]. Both the S64L and M153T mutations improve the folding of full-length green fluorescent protein (Tsien, Ann. Rev. Biochem.) and confer enhanced fluorescence to the intact, full-length protein (B.P. Cormack et al., Gene 173: 33-38). These mutations are a component of the YFP variant known as SEYFP (see Table 3). In example 3, described in detail

below, we directly compared a YFP PCA with the novel SEYFP PCA.

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In yet another example of engineering mutant fragments for PCA, we introduced the mutation F46L into fragment 1 of SEYFP, generating novel fragment we designated IFP[1], and we introduced the mutations V163A and S175G into fragment 2 of YFP, generating novel fragment IFP[2]. These mutations are a component of the YFP variant known as SEYFP-F46L ('Venus') in Table 3. The results demonstrate the ability to engineer a highly intense fluorescent PCA (IFP PCA) by engineering mutant fragments of fluorescent proteins.

In a fourth example of the invention, we demonstrated the ability to create PCAs with specific desired spectral properties by creating mutant polypeptide fragments. We created a cyan fluorescent PCA (CFP PCA) by synthesizing fragments with mutations conferring a spectral shift to the blue region. This invention provides fragments for generating a wide spectrum of PCAs through genetic engineering including green, yellow, blue-green, blue, cyan, orange-red and red variants with various intensities and signal maturation characteristics.

In a final example of the invention, we demonstrated multi-color PCAs in which a single fragment of a fluorescent reporter generates different fluorescent colors within the same cell, depending upon the amino acid sequence of the fragment with which it is paired.

## Example 1

## Creation of fluorescent protein-fragment complementation assays and the generation of mutant fragments for PCA

We sought to create two PCAs with different spectral properties starting with A. victoria GFP. First, GFP fragments were generated by PCR from a mammalian codon-optimized

version of GFP (pCMS-EGFP; Clontech). GFP[1] corresponded to amino acids 1 to 158 and GFP[2] to amino acids 159 to 239 of GFP. Second, fragments encoding a yellow variant of GFP (YFP PCA) were created by introducing the EYFP-specific mutations S65G, S72A into fragment 1 of GFP and the mutation T203Y into fragment 2 of GFP by PCR, resulting in fragments YFP[1] and YFP[2], respectively.

The fragments GFP[1], GFP[2], YFP[1], and YFP[2] were subcloned into a mammalian expression vector (pcDNA3.1Z, Invitrogen), which had previously been modified to incorporate the replication origin (oriP) of the Epstein Barr virus (EBV). The oriP allows episomal replication of these modified vectors in cell lines expressing the EBNA1 gene, such as HEK293E cells (293-EBNA, Invitrogen). Additionally, these vectors still retain the SV40 origin, allowing for episomal expression in cell lines expressing the SV40 large T antigen (e.g. HEK293T, Jurkat or COS) as well.

To test the activity and relative signal intensity of the GFP PCA versus the engineered YFP PCA, PCAs were created for three pairs of proteins that have previously been shown to interact in mammalian cells. These included the self-interaction of the tumor suppressor protein p53 (N.D. Lakin & S.P. Jackson, Oncogene 18: 7644-7655, 1999); the interaction of the papillomavirus E6 protein with p53 (B.A. Werness, A.J. Levine & P.M. Howley, Science 248: 76-79, 1990); and the interaction of the E6 protein with E6AP, a protein that mediates the interaction of E6 with p53 (J.M. Huibregtse, M. Scheffner & P.M. Howley, Mol. Cell. Biol. 13: 775-784, 1993). The full coding sequence for p53, E6 and E6AP was amplified by PCR from a sequence-verified full-length cDNA. The resulting PCR products were cleaned up by vacuum filtration (MultiScreen PCR, Amicon), digested with appropriate restriction enzymes to allow directional cloning, and fused in-frame to either the 5' or 3'-end of GFP[1], YFP[1], GFP[2] or

YFP[2] through a flexible linker encoding a 10-amino acid peptide (Gly.Gly.Gly.Gly.Ser)2 (SEQ ID NO:19). The use of a flexible linker between the gene of interest and the reporter fragment assures that the orientation and arrangement of the fusions is optimal to bring the fluorescent protein fragments into close proximity (J.N. Pelletier, F.-X. C.-Valois & S.W. Michnick, 1998, Proc Natl Acad Sci USA 95: 12141-12146). The orientations of the paired constructs was as follows: F1-linker-p53 with F2-linker-p53; F1-linker-E6 with E6AP-linker-F2; and F1-linker-E6 with F2-linker-p53, where F1 and F2 were the fragments of either GFP or YFP. DNAs from recombinant constructs were isolated using Qiagen Turbo BioRobot Prep kits (Qiagen, Chatsworth, CA) on a Beckman FX robotic workstation (Beckman Coulter, Fullerton, CA). Isolated DNAs were quantitated and then normalized to a concentration of 50 ng/µl.

Twenty-four hours prior to transfection, HEK293E cells were plated (20,000 cells per well) in 24-well plates coated with poly-lysine, then co-transfected with 0.5 micrograms of DNA using Fugene transfection reagent (Roche Diagnostics, Indianapolis, IN), as per the manufacturer's recommendations. Following 24 hrs of expression, cells were washed once with PBS and viewed on a Nikon TE-2000 microscope equipped with a HYQ-FITC filter cube (excitation: 460-500nm; emission:505-560 nm; dichroic mirror:505LP). Images were acquired with a CoolSnap HQ CCD camera. Figure 3 shows the results of fluorescence microscopy of GFP PCA vs. YFP PCA for the interactions of p53/p53, E6/E6AP and E6/p53. The reconstituted GFP or YFP signal could clearly be seen, and the subcellular localization of the complexes could be determined consistent with their known localizations. However, the YFP PCA signal was visually brighter than the GFP PCA signal for all three protein-protein complexes, demonstrating that the YFP mutations previously shown to enhance the signal intensity of the full-length protein were also effective in enhancing the intensity of the reassembled fragments. Moreover,

the excitation and emission maxima of the YFP PCA were nearly identical to that of the intact fluorescent protein YFP 10c (Table 2), suggesting that the complementary fragments are capable of folding and generating a chromophore with substantially the same properties as that generated by the intact protein.

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#### Example 2

# Alternative fragmentation sites in fluorescent proteins

In order to demonstrate that fluorescent protein fragments generated from alternative fragmentation sites - that are the subject of the invention - could be used in PCA, fluorescent protein PCAs based were created for a yellow (YFP) fluorescent protein (see FIG. 4). cDNAs encoding full-length proteins (Pdk2, 14-3-3 $\sigma$ , and the components of the NFKB heterodimer p50 and p65) were fused to either the N- or C-terminus of complementary YFP fragments YFP[1] and YFP[2], corresponding to fragmentation of the full length protein at positions shown in FIG. 1a and FIG. 1b as Gln 157, Lys 158 or Asp 173, where the indicated amino acid residue represents the C-terminus of the N-terminal reporter fragment designated as YFP[1]. Formation of 14-3-3/14-3-3 dimers was used to assess the ability of each PCA fragment pair to allow for the detection of protein-protein complexes. Pdk2-YFP[1]/Pdk2-YFP[2] was used as a negative PCA control. HEK293E cells were transiently transfected with 100ng of each construct pair, and total fluorescence was evaluated 48 hrs later on a Molecular Devices Gemini XS platereader. Each bar represents the mean fluorescence of triplicate measurements, with error bars representing 95% confidence limits. Mock-transfected cells (no DNA or a single DNA construct) are shown in yellow. Various fragment orientations and combinations were tested, since optimal detection of complex formation may be orientation-dependent. In this example, the Lys158 and Asp173 fragmentation sites allowed detection of 14-3-3/14-3-3 complexes in all possible fragment combinations. The Gln156 fragmentation site allowed detection of 14-3-3/14-3-3 complexes in both the NC and CC orientations. Fragment/gene orientations were as follows: NN=14-3-3-YFP[1]/14-3-3-YFP[2]; NC=14-3-3-YFP[1]/YFP[2]-14-3-3; CN=YFP[1]-14-3-3/14-3-3-YFP[2]; CC=YFP[1]-14-3-3/YFP[2]-14-3-3). The results demonstrate the utility of the protein engineering principles that are incorporated into this invention, showing that various fragment pairs are useful for PCA. The compositions that are the subject of the invention include various fragment pairs incorporating a wide range of mutations useful for PCA.

10 Example 3

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Mutant fragments generating a super-enhanced YFP PCA (SEYFP-PCA) and an intense fluorescent PCA (IFP PCA) with brighter signals than YFP PCA for biological applications

To further demonstrate that the spectral properties of a PCA can be influenced by engineering mutant fragments, we first engineered the F64L and M153T mutations of SEYFP into YFP[1] by PCR, creating novel fragment SEYFP[1]. Subcloning was performed as described for Example 1. Fusion constructs were prepared as described above, for the interacting protein kinases MEK and ERK. Specific mutations in each reporter fragment confirmed by sequencing are noted, and are designated relative to wtGFP as in Table 2 and 3.

For quantitative measurements of fluorescence intensity, each well of a 96-well polylysine coated plate was seeded with 15,000 HEK293E cells 24 hours prior to transfection. Cells were transfected with 100 ng of DNA in total per well with FuGene transfection reagent, using conditions recommended by the manufacturer. The amount of each fusion construct varied from 50 ng of each construct to as little as 0.1 ng of each construct, with the remaining DNA supplied by an empty 'carrier' vector (e.g. up to 98 ng of carrier DNA for 2 ng total of fusion construct

DNA). All transfections were performed in triplicate. Twenty-four or forty-eight hours after transfection, the cells were stained with a 1:300 dilution of Hoescht 33342 (Molecular Probes, Eugene, OR) for 10 minutes, then washed several times with Dulbecco's phosphate buffered saline, then overlaid with a small volume of Hank's Buffered Salt Solution. After a 90 minute incubation at 37°C, mean fluorescence intensity data for each well were acquired on a SpectraMax Gemini XS Plate reader (Molecular Devices), using an excitation wavelength of 485 nm, emission of 527 nm and cutoff of 515 nm. For each sample PCA, mean fluorescence intensity was calculated from triplicate measurements. Relative fold increase in fluorescence was determined by normalizing the mean fluorescence intensity for the test PCA to that of the negative control.

As shown in FIG. 5, the MEK/ERK protein-protein complex could be detected with either of the two fluorescent PCAs. However, the use of SEYFP[1] ( F64L and M153T) instead of YFP[1] enhanced the signal intensity two-to four-fold. With this particular mutant YFP PCA (left side of histogram), signal could be readily detected over background with only 2 ng total of 'test' DNA. Moreover, 10 ng DNA for the YFP PCA (right side of histogram) gave a barely detectable signal, whereas the equivalent DNA for the SEYFP PCA gave a signal nearly four times background (left side of histogram). The example demonstrates that mutations known to enhance the intensity of the intact protein confer a similar property on the reassembled fragments.

Fig. 6 shows fluorescence microscopy images of the same PCAs as in Fig. 5, demonstrating that the additional mutations of SEYFP[1] (panel a) enhance the signal intensity as detected by fluorescence microscopy, enabling improved discrimination of the subcellular location of the protein-protein complexes. HEK293E cells were transfected with 5 ng of each

fusion construct (plus 90 ng carrier DNA). Images were acquired 48 hrs later using the Discovery-1 automated image acquisition system, using a 20 ms exposure time, and FITC filter set. Protein-protein complexes could also be readily visualized with the YFP PCA (panel b) but were less intense.

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Since fragment 1 of these constructs contains all three of the amino acids that form the chromophore in the intact fluorescent protein, we also tested single mutant fragments YFP[1] and YFP[2] to ensure that individual fragments were incapable of generating a fluorescent signal. For panels c and d of Fig. 6, we transfected 50 ng of DNA from a single fusion construct with the indicated mutations. The left hand panel shows the fluorescence image; the right hand panel shows a DAPI stain of the cells, demonstrating that cells were present in the field that was imaged. Neither fragment alone, expressed as a fusion to protein Pdk2, gave a fluorescent signal. In subsequent analyses of over 6000 assays we found that under the experimental conditions we employed, the generation of a fluorescent PCA signal is dependent upon the interacting molecules. This is an important feature of the invention because it demonstrates that we are not tagging proteins with a fluorescent molecule. Rather, we are tagging proteins with polypeptide fragments which themselves are not fluorescent. The fluorescent signal is only generated upon interaction of the molecules to which the reporter fragments are fused. Interaction of the molecules of interest brings the reporter fragments into close proximity, allowing the fragments to fold together into an active structure capable of generating a fluorescent signal.

Figures 7a and 7b show the creation of yet another novel mutant fluorescent protein PCA allowing even greater sensitivity for biological applications. Mutations were selected based on the YFP variant designated SEYFP-F46L (Venus) in Table 3. These mutations have been shown to accelerate the maturation of the fluorescent signal in the intact protein (T. Nagai et al.,

2002, "A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications", Nature Biotech. 20: 87-90). PCR mutagenesis was employed to incorporate the additional mutations F46L into SEYFP[1], and V163A and S175G into YFP[2], resulting in novel fragments we designated IFP[1] and IFP[2].

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Formation of protein-protein complexes between the MAP kinase signaling proteins, MEK and ERK, was assessed with the novel IFP PCA by fusing MEK1 to the N-terminus of IFP[1] and ERK to the C-terminus of IFP[2]. As shown in FIGs. 7a and 7b, a titration series was performed wherein 100ng of total DNA was transfected per well, with the amount of DNA contributed by the PCA pair varying from 100ng down to 100pg, with the remaining DNA supplied by an empty 'carrier' vector. Fluorescence images were acquired 48 hours later on an SP Nikon fluorescence microscope using a HYQ-FITC filter cube (excitation: 460-500nm; emission:505-560 nm; dichroic mirror:505LP). Images were acquired with a CoolSnap HQ CCD camera with the indicated exposure times (in ms). Total fluorescence for each dilution of the MEK/ERK PCA was also quantified on a fluorescence platereader. Triplicate measurements for each dilution were made, and the mean fluorescence value was normalized to the mean fluorescence of a negative control PCA to determine the fold increase above the negative control, as shown in Figure 7b. Introduction of the additional mutations into the fragments of YFP greatly enhanced the fluorescent signal which could still be visualized (FIG. 7a) and quantified (FIG. 7b) even at 0.1 ng (100pg) of DNA. That level of DNA for the IFP PCA produced a significant signal above the negative control (1.5-fold increase). In contrast, to produce an equivalent fluorescence intensity with the YFP PCA, 10 ng DNA was needed.

The ability to identify the subcellular locations of protein-protein interactions enables high-content screening. For example, the trafficking of proteins within signaling pathways can

be seen. For example, we have used this approach with the IFP PCA described above to study the cytokine-induced translocation of the NFkB transcription complex of p65/p50 (FIG. 8). This protein-protein complex translocates from the cytoplasm to the nucleus in live cells in response to tumor necrosis factor. When p65 and p50 are tagged with complementary mutant fragments IFP[1] and IFP[2] respectively in transiently transfected cells, the fluorescent signal can be seen primarily in the cytoplasm in unstimulated cells 48 hours after transfection. Within 30 minutes of treatment of the TNF-responsive HEK cells with TNF-alpha, the fluorescent protein-protein complex moves predominantly to the nucleus.

The above examples demonstrate that mutations can be engineered into fluorescent protein fragments to confer specific desired properties for PCA. Accordingly, we have generated a number of novel fragments of fluorescent proteins incorporating previously described mutations of green fluorescent protein (see Table 2 and Table 3). These mutations have been engineered into fragments generated by fragmentation of fluorescent proteins at the sites depicted in Fig 1 and described in the above specification. Additionally, we have generated novel fragments at homologous fragmentation sites in coral fluorescent proteins (Table 4), in the monomeric red fluorescent protein (mRFP1) derived from DsRed (Table 5 and Table 6) and a kindling fluorescent protein (KFP1) derived from Anemonia sulcata (Table 7 and Table 8). The sequences of the novel fragments are shown in the Appendix prior to the claims of the present invention and are represented as SEQ ID NOS:20-1067 of the Sequence Listing and are the subject of the claimed invention. In general terms we refer to these as "mutant fragments". For the purposes of the invention, a "mutant fragment" is a fragment of a fluorescent protein that has one or more nucleotide or amino acid changes relative to the wild-type cDNA or protein.

#### Example 4

### Spectrally shifted PCAs

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Numerous examples of PCAs generating green fluorescent and yellow fluorescent signals have been described and demonstrated above. The invention described herein allows for PCAs generating a variety of spectral properties depending upon the amino acid sequence of the mutant fragments. In order to further demonstrate this principle, a PCA based on fragments of a cyan fluorescent protein was created to demonstrate blue fluorescence generated by a protein-protein interaction (FIG. 9). Two oligonucleotides corresponding to fragments of CFP were synthesized by Blue Heron Biotechnology (Bothell, WA). The resulting fragments were amplified by PCR to attach restriction sites and a flexible 10-aa linker for cloning into a pcDNA3-based expression vector, resulting in vectors containing CFP[1] (encoding aa 1-158 of ECFP) or CFP[2] (encoding aa 159-239 of ECFP) where the CFP had the amino acid sequence shown as ECFP in Table 3. The proteins Pdk2 and  $14-3-3\sigma$  were fused to the N-terminus of CFP[1] and CFP[2], respectively, while the subunits of the NFkB heterodimer p50 and p65 were fused to the Cterminus of the CFP fragments. The construct pairs 14-3-3\sigma/14-3-3\sigma, p65/p50 and the Pdk2/Pdk2 negative control were transiently transfected into HEK293T cells, and fluorescence microscopy was performed after 48 hours. Fluorescence images were acquired on an SP Nikon fluorescence microscope using a Chroma CFP filter (excitation: 426-446nm; emission: 460-500nm; dichroic mirror:455LP). Images were acquired with a CoolSnap HQ CCD camera with exposure times of 1-5 sec, as shown in Figure 8. The results show that mutations causing a spectral shift in the intact fluorescent protein can be engineered into fragments for PCA, resulting in a PCA generating a blue fluorescent signal with utility for biological applications.

#### Example 5

#### Multi-color PCAs

The availability of a suite of fluorescent protein PCAs enables the construction of multicolor PCAs for a variety of biology, biotechnology, drug discovery and diagnostic applications. Such multi-color PCAs are another aspect of the invention.

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For example, a 'generic' F2 polypeptide fragment could be combined with multiple distinct F1 mutant fragments in order to detect two, three, four or more bimolecular events simultaneously. This can be achieved by fragmenting a fluorescent protein in such a way that F1 contains all the amino acid residues necessary for chromophore formation when complemented by F2. Two or more mutant fragments of F1 are then created. For example, mutant F1 fragments that are capable of reconstituting either a green, yellow, cyan, blue or red signal can be generated. If F2 is fused to molecule A, and the mutant F1 fragments are fused separately to molecules B, C, D, E and F respectively, the interactions of A with B, A with C, A with D, A with E and A with F can all be tested simultaneously by testing for a fluorescence signal at the 5 different wavelengths that are generated by fragment complementation.

We demonstrated the principles of multi-color PCA in living cells by using the ability of the NFκB p65 subunit to form complexes with the p50 subunit, and also with the protein IkBα, as a model system. In resting cells, IkBα binds to NFkB and retains the complex in the cytoplasm. Thus, p65 forms cytoplasmic protein-protein complexes with p50 and also with IκBα We co- transfected HEK293T cells simultaneously with three PCA constructs: CFP[1]-p50; CFP[2]-p65; and IκBα-YFP[1]. Fluorescence images were acquired with an SP Nikon fluorescence microscope using a Chroma CFP filter (excitation: 426-446nm; emission:460-

500nm; dichroic mirror:455LP), and a FITC filter (excitation: 460-500nm; emission:505-560 nm; dichroic mirror:505LP). 16-bit monochrome images were acquired with a CoolSnap HQ CCD camera. CFP and FITC images for each PCA were subsequently pseudocolored and overlaid using Metamorph software (Molecular Devices). If a protein-protein complex forms between p50 and p65, the CFP[1] fragment should complement the CFP[2] fragment, producing blue fluorescence. Alternatively, if a protein-protein complex forms between IkBα and p65, the YFP[1] fragment should complement the CFP[2] fragment, producing a yellow fluorescence. As shown in FIG. 10, both p65/p50 (blue) and IkBα/p65 (yellow) complexes could be detected in the cytoplasm as expected. Cells displaying a lighter yellow (almost white) cytoplasmic staining pattern are expressing both p65/p50 and IkBα/p65 complexes. The ability to construct multicolor PCAs allows for the detection and quantification of multiple distinct protein-protein complexes within the same cells.

# Additional Applications of Fluorescent Protein PCAs

The many practical applications of this invention include high-content and high-throughput assays in living cells, cell lysates, or in vitro formats. The applications of the invention include the detection of pathway activation and pathway 'switching' in living cells by agonists, antagonists and inhibitors. The translocation or trafficking of proteins from one subcellular compartment to another can be followed; if protein A initially binds to protein B at the cell membrane and generates a yellow fluorescent signal, and then moves to the cell nucleus and binds to protein C and generates a cyan fluorescent signal, the ratio of cyan to yellow can be used as a detector of the activation of the translocation event. Moreover, there are many applications for fluorescent protein PCAs in diagnostics and nanotechnology. For example,

mutant F1 fragments could be bound to a solid surface array, each one as a fusion with a different antibody, which could be used to detect the presence of specific antigens in a sample. The applications of multicolor PCAs include rapid, multicolor diagnostics for biowarfare agents. Such multicolor PCAs are made possible by the novel mutant fragments that are the subject of the present invention.

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The cells can be studied in vitro in a variety of formats including tissue culture plates, microtiter plates, or slide formats. The cells harboring PCA constructs can also be studied in vivo. For example, suitable cultured cells stably expressing a particular PCA can be grown as ascites in living animals, or introduced into nude mice to form tumors. Alternative, transgenic mice harboring the PCA constructs can be made. The protein-protein complexes within the animal can then be studied by whole animal imaging systems, for example, those supplied by Xenogen (Alameda, CA) or Anti-Cancer (San Diego, CA). All the PCAs presented here, and the various intense yellow and red fluorescent PCAs, will be particularly useful for PCA in vivo. In vivo PCA applications include the ability to generate a PCA that responds in vivo to the consumption or injection of a drug by the animal. Applications to pre-clinical drug development include the ability to perform ADME studies (absorption, distribution, metabolism or excretion of a drug) in live animals without sampling blood or urine. For example, if a drug causes an increase or decrease in a specific protein-protein complex within a cell in the live animal, the fluorescent signal can be acquired at various times after drug administration which will allow estimation of the pharmacokinetic and pharmacodynamic properties of the drug in whole animals.

Finally, the availability of a wide range of complementing mutant fragments of fluorescent proteins enables empirical testing for mutant fragment combinations that are

particularly useful for PCA. It is likely that this combinatorial feature of PCA will enable the generation of a large number of novel assays with a range of colors, intensities, combinations and physical properties for use in drug screening, target validation, ADME, and diagnostics applications.

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The entire contents including the references cited therein of the following patents and publications are incorporated by reference in their entirety for all purposes to the same extent as if each individual patent, patent application or publication were so individually denoted.

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The sequences of the novel fragments are shown in the Appendix below and are represented as SEQ ID NOS:20-1067 of the Sequence Listing and are the subject of the claimed invention.

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## APPENDIX

5		F1 (a			of w	GF)	P) pa	os. :	l Me	t re	nove	đ				
10					gaa Glu 5											48
10					gtt Val											96
15	_		_	_	aca Thr										:	114
20		F2 (a			of 23	wt (	GFP)	+ Me	et @	pos	sitio	on 1		·		
25					acc Thr 5											48
30					aca Thr											96
30					cca Pro										:	144
35					ggt Gly										:	192
40					aag Lys										2	240
45					atc Ile 85										2	288
50	aac Asn				cac His										3	336
30					gac Asp										3	384
55	Ile				att Ile										4	132

5								cct Pro					480
								tcg Ser 170					528
10								gta Val					576
15				cta Leu									600
20		(aa :		EYFP)	) - N	Met @	pos	sitio	on 1			٠	
25								999 Gly 10				gtc Val	48
20								aag Lys					96
30				acc Thr									117
35		(aa 4 NOS:2		E EYI	FP) +	⊦ Met	: @ <u>r</u>	posit	ion	1	•		
40			Leu					tgc Cys 10					48
45								ttc Phe					96
								cgg Arg					144
50								cgc Arg					192
55								gtg Val					240

5				gag Glu								288
				aag Lys	_				_		_	336
10				aag Lys	_	_		-			_	384
15				gag Glu								432
20				atc Ile 150								480
25				cag Gln				_			_	528
				ctg Leu								576
30				ctg Leu								600
35	YFPI SEQ	F2A ID 1	5L mi 28 &	ion								
40				aag Lys								48
45				gtg Val								96
				cac His								144
50				gtc Val								192
55				cgc Arg 70								240

											ttc Phe						288
5											aac Asn						336
10											aag Lys						384
15											ctc Leu						432
20											ctg Leu 155						480
											gac Asp					cgc Arg	528
25											gcc Ala						576
30		_	_	gag Glu	_		_										597
35	YFPI SEQ			16L m 30 &		ion	+ Me	et @	post	ion	1						
40						Leu	Lys	Leu		Cys	acc Thr						48
45											ggc Gly						96
		Ala									cac His						144
50											acc Thr						192
55	gac Asp 65	ggc Gly	aac Asn	tac Tyr	aag Lys	acc Thr 70	Arg	gcc Ala	gag Glu	gtg Val	aag Lys 75	ttc Phe	gag Glu	ggc Gly	gac Asp	acc Thr 80	240

						gag Glu							:	288
5						aag Lys								336
10			_	_	_	aag Lys	_	_		_		_	:	384
15						gag Glu							4	132
20						atc Ile 150							4	480
						cag Gln								528
25						ctg Leu							į	576
30						ctg Leu		_					(	500
35	YFPI SEQ			16L/E 32 &		muta	ation	ıs						
40						aag Lys								48
45						gtg Val								96
						cac His							<u>:</u>	144
50		Pro				gtc Val							<u>:</u>	192
55						cgc Arg 70							2	240

						ctg Leu										288
5						ctg Leu										336
10						cag Gln										384
15						gac Asp										432
20		Asn				ggc Gly 150										480
			_		_	tcc Ser	_	_	-		_			_	_	528
25						ctg Leu										576
30						tac Tyr										597
35	YFPI SEQ		F4 NOS:3		764L 35	mut	atio	ons -	⊦ Met	2 @ p	posit	. 1				
40		Gly	Lys	Leu	Thr	ctg Leu	Lys	Leu	Ile	Cys	Thr		Lys			48
45						ctc Leu										96
						gac Asp										144
50						tac Tyr										192 <sup>.</sup>
55						acc Thr 70										240

						gag Glu							288
5						aag Lys							336
10						aag Lys							384
15						gag Glu							432
20						atc Ile 150							480
						cag Gln							528
25						ctg Leu							576
30						ctg Leu							600
35	YFPI SEQ			54L m 36 &		ion							
40		_	_		_	aag Lys		_		 _	_	~ ~	48
45						gtg Val							96
						cac His							144
50						gtc Val							192
55						cgc Arg 70							240

						ctg Leu											288
5						ctg Leu											336
10						cag Gln											384
15	_					gac Asp		_		_		_	_			_	432
20		Asn				ggc Gly 150											480
						tcc Ser											528
25						ctg Leu											576
30						tac Tyr											597
35	YFPI SEQ			54L n 38 &		ion	+ Me	et @	posi	tion	1 1						
40			Lys		Thr	ctg Leu			Ile								48
45						ctc Leu				_				_	_	_	96
		Ala				gac Asp						-			_		144
50						tac Tyr											192
55						acc Thr 70											240

						gag Glu								288
5						aag Lys								336
10						aag Lys								384
15		_				gag Glu	_		_	 _	_	_		432
20						atc Ile 150								480
						cag Gln								528
25						ctg Leu								576
30			-	_	_	ctg Leu		_						600
35	YFPI SEQ			79R π 10 &		cion								
40			_		_	aag Lys			_		 _	_		48
45						gtg Val								96
						cac His								144
50						gtc Val								192
55						cgc Arg 70								240

												gac Asp			288
5												aac Asn 110			336
10												ttc Phe			384
15												cac His			432
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												gag Glu	cgc Arg		528
25												atc Ile 190			576
30			gac Asp 195								•				597
35	YFPI SEQ		K7 108:4		tion	+ Me	et @	posi	it. 1	L					
40			Lys	Thr				Ile				aag Lys			48
45												ctg Leu 30			.96
												ttc Phe			144
50												ttc Phe			192
55												ggc Gly		,	240

				cgc Arg					Gly						288
5				999 Gly 100		_	_					_		_	336
10				gcc Ala											384
15				aac Asn											432
20				acc Thr											480
				agc Ser											528
25				atg Met 180											576
30				gac Asp											600
35	YFPI SEQ			56F m		ion									
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45				acc Thr 20											96
				ccc Pro											144
50				ggc Gly											192
55				aag Lys											240

			cgc Arg														288
5			ggg Gly		_	_						_			_		336
10			gcc Ala 115														384
15			aac Asn														432
20		Asn	acc Thr														480
			agc Ser													cgc Arg	528
25			atg Met								_	_					576
30			gac Asp 195														597
35	YFPI SEQ		Y6 105:4			ion	+ Me	et @	posi	it. 1	L .						
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45			tgg Trp														96
			cgc Arg 35														144
50			ccc Pro														192
55			aac Asn														240

					gag Glu							288
5			_		aag Lys	_			_			336
10					aag Lys							384
15		_			gag Glu	_	 _	 _	_	_		432
20					atc Ile 150							480
					cag Gln							528
25					ctg Leu							576
30					ctg Leu							600
35	YFPI SEQ			59Κ π 18 &	ion							•
40					aag Lys							48
					gtg Val							96
45					cac His							144
50					gtc Val							192
55					cgc Arg 70							240

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5						ctg Leu											3	36
10						cag Gln											3	84
15						gac Asp											4	32
20		Asn				ggc Gly 150											4	80
						tcc Ser											5	28
25						ctg Leu											5	76
30						tac Tyr		·									5	97
35	YFPI SEQ			59K r 50 &		ion	+ M6	et @	posi	it. 1	L ·							
35 40	SEQ atg	ID N	10S:9	50 & ctg	51 acc	cion ctg Leu	aag	ttc	atc	tgc	acc			_	_			48
	SEQ atg Met 1	ID N ggc Gly ccc	aag Lys tgg	ctg Leu ccc	acc Thr 5	ctg	aag Lys gtg	ttc Phe	atc Ile	tgc Cys 10	acc Thr	Thr tac	Gly	Lys	Leu 15 aag	Pro tgc		48 96
	sEQ atg Met 1 gtg Val	ggc Gly	aag Lys tgg Trp	ctg Leu ccc Pro 20	acc Thr 5 acc Thr	ctg Leu ctc	aag Lys gtg Val	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	Thr tac Tyr	Gly ggc Gly ttc	Lys ctg Leu 30	Leu 15 aag Lys aag	Pro tgc Cys	1	
40	SEQ atg Met 1 gtg Val ttc Phe	ggc Gly ccc Pro gcc Ala	aag Lys tgg Trp cgc Arg 35	ctg Leu ccc Pro 20 tac Tyr	acc Thr 5 acc Thr ccc Pro	ctg Leu ctc Leu	aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	Thr tac Tyr gac Asp	Gly  ggc Gly  ttc Phe 45	Lys  ctg Leu 30  ttc Phe	Leu 15 aag Lys aag Lys	tgc Cys tcc Ser		96

				cgc Arg					Gly								288
5				999 Gly 100													336
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15				aac Asn													432
20				acc Thr													480
				agc Ser												aag Lys	528
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35 40	SEQ ggc	ID N	ios:		53 ctg	aag	ttc	atc	tgc								48
40	SEQ ggc Gly 1 ccc	ID Naag Lys tgg	ctg Leu	52 & acc	53 ctg Leu 5	aag Lys gtg	ttc Phe	atc Ile acc	tgc Cys ttc	Thr 10 ggc	Thr	Gly ggc	Lys	Leu	Pro 15 tgc	Val ttc	48 96
	ggc Gly 1 ccc Pro	aag Lys tgg Trp	ctg Leu ccc Pro	acc Thr acc	ctg Leu 5 ctc Leu	aag Lys gtg Val	ttc Phe acc Thr	atc Ile acc Thr	tgc Cys ttc Phe 25	Thr 10 ggc Gly cac	Thr tac Tyr	Gly ggc Gly ttc	Lys ctg Leu	Leu atg Met 30	Pro 15 tgc Cys	Val ttc Phe	
40	ggc Gly 1 ccc Pro gcc Ala	aag Lys tgg Trp cgc Arg	ctg Leu ccc Pro tac Tyr 35	acc Thr acc Thr 20	53 ctg Leu 5 ctc Leu gac Asp	aag Lys gtg Val cac His	ttc Phe acc Thr atg Met	atc Ile acc Thr aag Lys 40	tgc Cys ttc Phe 25 cgg Arg	Thr 10 ggc Gly cac His	tac Tyr gac Asp	Gly ggc Gly ttc Phe	ctg Leu ttc Phe 45	atg Met 30 aag Lys	Pro 15 tgc Cys tcc ser	Val ttc Phe gcc Ala	96

						ctg Leu											288
5						ctg Leu											336
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15						gac Asp											432
20		Asn				ggc Gly 150											480
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35	citi SEQ atg Met 1 gtg Val	rinel ID 1 ggc Gly ccc Pro gcc Ala	195 F2A NOS:5 aag Lys tgg Trp	Ctg Leu Ccc Pro 20	3L/ 0 55 acc Thr 5 acc Thr	Q69K ctg Leu ctc	muta aag Lys gtg Val	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	acc Thr tac Tyr	ggc Gly ggc Gly	Lys ctg Leu 30	Leu 15 atg Met	Pro tgc Cys	
35 40	citi SEQ atg Met 1 gtg Val ttc Phe	rinel ID 1 ggc Gly ccc Pro gcc Ala	195 F2A NOS:S aag Lys tgg Trp cgc Arg 35 ccc	ctg Leu ccc Pro 20 tac Tyr	BL/ (55 acc Thr 5 acc Thr ccc Pro	069K ctg Leu ctc Leu	muta aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	acc Thr tac Tyr gac Asp	ggc Gly ggc Gly ttc Phe 45	ctg Leu 30 ttc Phe	Leu 15 atg Met aag Lys	tgc Cys tcc Ser	96

			gag Glu											288
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10			aag Lys											384
15			gag Glu											432
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25			ctg Leu											576
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40			aag Lys											48
45			gtg Val											96
			cac His											144
50			gtc Val											192
55			cgc Arg 70											240

			_		 _	_	ggc Gly		_		_		-			288
5							tac Tyr									336
10							aac Asn 120									384
15							agc Ser									432
20		Asn					ggc Gly									480
							ctg Leu								cgc Arg	528
25							ttc Phe									576
30					tac Tyr											597
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40							ttc Phe									48
							acc Thr									96
45							atg Met 40									144
50				_		_	cag Gln		_					_	-	192
55							gcc Ala								acc Thr 80	240

			gag Glu							:	288
5			aag Lys	_			_		_		336
10			aag Lys							;	384
15			gag Glu							4	432
20			atc Ile 150							4	480
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50			gtc Val							<del>.</del>	192
55			cgc Arg 70							2	240

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5					ctg Leu										336
10					cag Gln										384
15					gac Asp										432
20	Asn				ggc Gly 150										480
					tcc Ser									cgc Arg	528
25					ctg Leu										576
30					tac Tyr										597
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40	Gly	Lys	Leu	Thr	ctg Leu	Lys	Phe	Ile		Thr		Lys			48
45					ctc Leu				_			_	_	_	96
	Ala				gac Asp	His									144
50					tac Tyr										192
55					acc								gac Asp		240

						gag Glu											288
5						aag Lys											336
10						aag Lys											384
15						gag Glu											432
20						atc Ile 150											480
						cag Gln											528
25						ctg Leu											576
	at a																600
30				gac Asp		Leu											800
30 35	Leu	Gly F2A	Met 195	Asp	Glu		Tyr	Lys						·			600
	CFP SEQ ggc	Gly F2A ID N	Met 195 , Ye NOS:6	Asp 56W 54 & acc	muta 65	Leu	Tyr 1 ttc	Lys 200 atc									48
35	CFP SEQ ggc Gly 1	F2A ID N aag Lys	Met 195 , Ye JOS:6 ctg Leu	Asp 66W 64 & acc Thr	muta 65 ctg Leu 5	Leu ation aag	Tyr ttc Phe	Lys 200 atc Ile	Cys ttc	Thr 10 ggc	Thr tgg	Gly ggc	Lys ctg	Leu	Pro 15 tgc	Val	
35	CFP SEQ Gly 1 CCC Pro	Gly F2A ID N aag Lys tgg Trp	Met 195 , Ye NOS:6 ctg Leu ccc Pro	Asp 66W 64 & acc Thr acc Thr 20	muta 65 ctg Leu 5 ctc Leu	Leu atior aag Lys gtg	Tyr  ttc Phe  acc Thr	Lys 200 atc Ile acc Thr	Cys ttc Phe 25	Thr 10 ggc Gly cac	Thr tgg Trp	Gly ggc Gly ttc	Lys ctg Leu ttc	cag Gln 30	Pro 15 tgc Cys	Val ttc Phe	48
35 40	CFP SEQ ggc Gly 1 ccc Pro	F2A ID N aag Lys tgg Trp cgc Arg	Met 195 , Ye JOS: 6 ctg Leu ccc Pro tac Tyr 35 gaa	Asp 66W 64 & acc Thr acc Thr 20 ccc Pro	muta 65 ctg Leu 5 ctc Leu gac Asp	Leu atior aag Lys gtg Val	Tyr  ttc Phe  acc Thr  atg Met  cag	atc Ile acc Thr aag Lys 40	ttc Phe 25 cgg Arg	Thr 10 ggc Gly cac His	tgg Trp gac Asp	Gly ggc Gly ttc Phe	Lys ctg Leu ttc Phe 45	cag Gln 30 aag Lys	Pro 15 tgc Cys tcc ser	Val ttc Phe gcc Ala	48 96

						ctg Leu											288
5						ctg Leu											336
10						cag Gln											384
15						gac Asp											432
20						ggc Gly 150											480
						tcc Ser			_		_				_	_	528
25						ctg Leu											576
30					-	tac Tyr	_										597
35	CFP		, Y6 10s:6			ation	1 + N	1et @	) pos	sit.	1 .						
40	SEQ atg	ID 1	105:6 aag	66 & ctg	67 acc	atior ctg Leu	aag	ttc	atc	tgc	acc						48
35 40	sEQ atg Met 1 gtg	ID N	aag Lys tgg	ctg Leu ccc	acc Thr 5	ctg	aag Lys gtg	ttc Phe	atc Ile	tgc Cys 10 ttc	acc Thr	Thr tgg	Gly	Lys	Leu 15 cag	Pro tgc	48 96
40	sEQ atg Met 1 gtg Val	ggc Gly	aag Lys tgg Trp	ctg Leu ccc Pro 20	acc Thr 5 acc Thr	ctg Leu ctc	aag Lys gtg Val cac	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	Thr tgg Trp	Gly ggc Gly ttc	Lys ctg Leu 30	Leu 15 cag Gln aag	Pro tgc Cys	
40	sEQ atg Met 1 gtg Val ttc Phe	ggc Gly ccc Pro	aag Lys tgg Trp cgc Arg 35	ctg Leu ccc Pro 20 tac Tyr	acc Thr 5 acc Thr ccc Pro	ctg Leu ctc Leu	aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	tgg Trp gac Asp	Gly  ggc Gly  ttc Phe 45	ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	tgc Cys tcc Ser	96

						gag Glu											288
5						aag Lys											336
10						aag Lys											384
15						gag Glu	_				_		_	_			432
20						atc Ile 150											480
						cag Gln											528
25						ctg Leu											576
30 .						ctg Leu		_									600
30	Leu	Gly F2A	Met 195		Glu	_	Tyr	Lys									600
35	CFP SEQ ggc	Gly F2A ID N	Met 195 , Se NOS:6	Asp 55A 58 & acc	muta 69 ctg	Leu	Tyr ttc	Lys 200	_				_	_			48
	CFP SEQ ggc Gly 1	F2A ID N aag Lys	Met 195 , se JOS: e ctg Leu	Asp 55A 68 & acc Thr	muta 69 ctg Leu 5	Leu ation aag	Tyr ttc Phe	Lys 200 atc Ile	Cys	Thr 10 gcc	Thr tac	Gly	Lys	Leu	Pro 15 tgc	Val	
35	CFP SEQ Gly 1 ccc Pro	Gly F2A ID 1 aag Lys tgg Trp	Met 195 , Se NOS: 6 ctg Leu ccc Pro	Asp 55A 68 & acc Thr acc Thr 20	muta 69 ctg Leu 5 ctc Leu	Leu ation aag Lys gtg	Tyr  ttc Phe  acc Thr	Lys 200 atc Ile acc Thr	Cys ttc Phe 25	Thr 10 gcc Ala cac	Thr tac Tyr	Gly ggc Gly ttc	Lys ctg Leu	Leu cag Gln 30	Pro 15 tgc Cys	Val ttc Phe	48
35 40	CFP SEQ Gly 1 CCC Pro	F2A ID 1 aag Lys tgg Trp cgc Arg	Met 195 , Se JOS: C Ctg Leu CCC Pro tac Tyr 35 gaa	Asp 55A 68 & acc Thr acc Thr 20 ccc Pro	muta 69 ctg Leu 5 ctc Leu gac Asp	Leu ation aag Lys gtg Val	Tyr  ttc Phe  acc Thr  atg Met  cag	atc Ile acc Thr aag Lys 40	Cys ttc Phe 25 cgg Arg	Thr 10 gcc Ala cac His	tac Tyr gac Asp	Gly ggc Gly ttc Phe	Lys  ctg Leu  ttc Phe 45	cag Gln 30 aag Lys	Pro 15 tgc Cys tcc ser	Val ttc Phe gcc Ala	48 96

						ctg Leu											288
5						ctg Leu											336
10						cag Gln											384
15						gac Asp											432
20		Asn				ggc Gly 150											480
						tcc Ser											528
25	_		_	_	_	ctg Leu					_	_					576
	aac	ato	aac	aaa	cta	tac	aao										597
30						Tyr											
35	Gly	Met F2A	Asp 195	Glu	Leu		Lys	1et @	) pos	sit 1	L						
	Gly CFP SEQ atg	Met F2A ID 1	Asp 195 , Se NOS:	Glu 55A 70 & ctg Leu	muta 71	Tyr	Lys n + N aag	ttc	atc	tgc	acc						48
35	CFP SEQ atg Met 1	F2A ID 1 ggc Gly	Asp 195 , Se NOS: aag Lys	Glu 65A 70 & ctg Leu ccc	muta 71 acc Thr 5	Tyr ation	Lys a + N aag Lys gtg	ttc Phe acc	atc Ile	tgc Cys 10 ttc	acc Thr	Thr tac	Gly	Lys	Leu 15 cag	Pro tgc	
35 40	CFP SEQ atg Met 1 gtg Val	F2A ID 1 ggc Gly ccc Pro	Asp 195 , se NOS: aag Lys tgg Trp	Glu  55A  70 &  ctg Leu  ccc Pro 20  tac	muta 71 acc Thr 5 acc Thr	Tyr  ation  ctg  Leu  ctc	Lys aag Lys gtg Val cac	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr gcc Ala	Thr tac Tyr gac	Gly ggc Gly ttc	Lys ctg Leu 30	Leu 15 cag Gln aag	Pro tgc Cys	48
35 40	CFP SEQ atg Met 1 gtg Val ttc Phe	F2A ID N ggc Gly ccc Pro	Asp 195 , Se NOS: aag Lys tgg Trp cgc Arg 35	Glu  55A  70 &  ctg Leu  ccc Pro 20  tac Tyr	muta 71 acc Thr 5 acc Thr	Tyr ation ctg Leu ctc Leu gac	Lys aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr gcc Ala cac His	tac Tyr gac Asp	Gly  ggc Gly  ttc Phe 45	ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	tgc Cys tcc Ser	48

					gag Glu			Gly						288
5					aag Lys									336
10					aag Lys									384
15					gag Glu									432
20					atc Ile 150									480
		_	_		cag Gln		_	_	_	_		 _	•	528
25					ctg Leu									576
30					ctg Leu						,			600
35			55A/\ 72 &		/T203	3 <b>Y</b> r	nutat	ions	5				,	
40				Leu	aag Lys			Cys						48
45					gtg Val									96
					cac His									144
50					gtc Val									192
55					cgc Arg 70									240

				ctg Leu										288
5				ctg Leu										336
10				cag Gln										384
15				gac Asp										432
20				ggc Gly 150										480
				tcc Ser										528
25				ctg Leu										576
30			_	tac Tyr	_									597
35	CGFI SEQ		55A/\ 74 &	/T203	BY r	nutat	ions	5 + N	1et @	pos	s. 1			
40				ctg Leu								-	-	48
				ctc Leu										96
45				gac Asp										144
50				tac Tyr										192
													gac	240

			gag Glu											288
5			aag Lys											336
10			aag Lys											384
15			gag Glu											432
20			atc Ile 150											480
			cag Gln									aag Lys		528
25			ctg Leu											576
30			ctg Leu											600
35		54L/S 76 &	/Y66V	V/M15	53T/V	/163 <i>I</i>	A/T2(	03Y n	nutat	cions	3		·	
40		Thr	aag Lys											48
45			gtg Val											96
			cac His	Met										144
50			gtc Val											192
55			cgc Arg 70									ctg Leu 80		240

						ctg Leu												288
5						ctg Leu												336
10						cag Gln												384
15						gac Asp												432
20						ggc Gly 150												480
						tcc Ser												528
25						ctg Leu												576
30						tac Tyr												597
35		P F21	A, F64	lL/Se	55T/Y	766W/	/M153	BT/V1	163A,	/T203	SY mι	ıtati	ions	+ M∈	et @	pos.	1	
40						ctg Leu												48
	ata																	
45						ctc Leu												96
45	Val ttc	Pro gcc	Trp	Pro 20 tac	Thr		Val cac	Thr atg	Thr 25 aag	Leu cgg	Thr cac	Trp gac	Gly ttc	Leu 30 ttc	Gln aag	Cys		96
50	ttc Phe gcc	Pro gcc Ala atg	Trp cgc Arg 35 ccc	Pro 20 tac Tyr	Thr ccc Pro	Leu gac	val cac His	Thr atg Met 40 cag	Thr 25 aag Lys gag	Leu cgg Arg	Thr cac His	Trp gac Asp atc	ttc Phe 45	Leu 30 ttc Phe	Gln aag Lys aag	tcc ser		

				gag Glu										288
5		_		aag Lys	_						_		-	336
10				aag Lys										384
15				gag Glu										432
20				atc Ile 150										480
		_	_	cag Gln		_	_	-		_			aag Lys	528
25				ctg Leu										576
30				ctg Leu										600
35			1/S65 30 &	1\W6	N146:	I/M15	53T/V	/163 <i>I</i>	A mut	atio	ons			
40				aag Lys										48
45				gtg Val										96
				cac His										144
50				gtc Val										192
55				cgc Arg 70										240

						ctg Leu											288
5						ctg Leu											336
10						cag Gln											384
15						gac Asp											432
20						ggc Gly 150											480
						tcc Ser											528
25						ctg Leu											576
30						tac Tyr											597
35				A,F46 32 &		55 <b>T/</b> Y	766W/	/N146	5I/M1	L53T,	/V163	3 <b>A</b> mu	ıtati	lons	+ Me	et @ pos	3. 1
40						ctg Leu											48
45						ctc Leu											96
						gac Asp											144
50						tac Tyr											192
55	gac Asp 65	ggc Gly	aac Asn	tac Tyr	aag Lys	acc Thr 70	cgc Arg	gcc Ala	gag Glu	gtg Val	aag Lys 75	ttc Phe	gag Glu	ggc Gly	gac Asp	acc Thr 80	240

					gag Glu											288
5					aag Lys											336
10					aag Lys											384
15					gag Glu											432
20					atc Ile 150											480
20					cag Gln											528
25					ctg Leu											576
30					ctg Leu						•					600
35			16I m 34 &		ion											
40	Lys	Leu	Thr	Leu	aag Lys	Phe	Ile	Cys	Thr	Thr	Gly	Lys	Leu	Pro		48
45					gtg Val											96
	Arg				cac His											144
50					gtc Val											192
55					cgc Arg 70											240

					ctg Leu									288
5					ctg Leu									336
10					cag Gln									384
15					gac Asp									432
20					ggc Gly 150									480
					tcc Ser									528
25					ctg Leu									576
30					tac Tyr									597
35		, N14 NOS:8			ion	+ M6	et @	pos	it. I	L				
40	Gly	Lys	Leu	Thr	ctg Leu	Lys	Phe	Ile	Cys	Thr		Lys		48
45					ctc Leu									96
					gac Asp									144
50					tac Tyr									192
55					acc Thr									240

				gag Glu									288
5		_		aag Lys	_					_		_	336
10				aag Lys									384
15				gag Glu									432
20				atc Ile 150									480
		_	_	cag Gln		_	_	_	_			_	528
25				ctg Leu									576
30		_	_	 ctg Leu		_							600
35			53T r 38 &	cion									
40			Thr	aag Lys									48
45				gtg Val									96
	Arg			cac His									144
50				gtc Val									192
55				cgc Arg 70								ctg Leu 80	240

					ctg Leu										288
5	_			_	ctg Leu						_		_		336
10		_	_	_	cag Gln	_				_	_		_		384
15					gac Asp										432
20					ggc Gly 150										480
					tcc Ser										528
25					ctg Leu									٠	576
30					tac Tyr										597
35			53T r 90 &		cion	+ Me	et @	posi	it. 1	L					
40			Leu		ctg Leu			Ile							48
45					ctc Leu										96
	_	_			gac Asp		_	_			_		_		144
50					tac Tyr										192
55					acc Thr 70										240

	_			_		gag Glu	_	_			_		_		_		288
5			_			aag Lys							_			_	336
10						aag Lys											384
15						gag Glu											432
20		Gln				atc Ile 150											480
			_	_		cag Gln			_	_						aag Lys	528
25						ctg Leu											576
		ggc	atg	gac	gag	ctg	tac										600
30	Leu	Gly	Met 195	Asp	Glu	Leu	Tyr	Lys 200									
35	CFP	F2A	195 ,N14		M153	Leu 3T mi		200									
	CFP SEQ ggc	F2A ID 1	,N14 NOS:9	16I/ 92 & acc	M153 93 ctg Leu		ıtati	200 lons	Cys								48
35	CFP SEQ ggc Gly 1	F2A ID N aag Lys	,N14,NOS:S	46I/ 92 & acc Thr	M153 93 ctg Leu 5	3T mu aag	ttc Phe	200 lons atc Ile acc	Cys	Thr 10 ggc	Thr tac	Gly	Lys	Leu	Pro 15 tgc	Val	.96
35 40	CFP SEQ ggc Gly 1 ccc Pro	F2A ID N aag Lys tgg Trp	,N14,NOS:S	acc Thr acc Thr 20	M153 93 ctg Leu 5 ctc Leu	aag Lys gtg Val cac	ttc Phe acc Thr atg Met	200 cons atc Ile acc Thr	Cys ttc Phe 25 cgg	Thr 10 ggc Gly cac	Thr tac Tyr	Gly ggc Gly ttc	Lys ctg Leu ttc	cag Gln 30	Pro 15 tgc Cys	Val ttc Phe	
35 40	CFP SEQ ggc Gly 1 ccc Pro	F2A ID N aag Lys tgg Trp cgc Arg	,N14 NOS:S  ctg Leu  ccc Pro  tac Tyr 35 gaa	acc Thr acc Thr 20 ccc Pro	M153 93 ctg Leu 5 ctc Leu gac Asp	aag Lys gtg Val cac	ttc Phe acc Thr atg Met	atc Ile acc Thr aag Lys 40 gag	ttc Phe 25 cgg Arg	Thr 10 ggc Gly cac His	tac Tyr gac Asp	Gly ggc Gly ttc Phe	ctg Leu ttc Phe 45	cag Gln 30 aag Lys	Pro 15 tgc Cys tcc ser	Val ttc Phe gcc Ala	.96

						ctg Leu											288
5						ctg Leu											336
10						cag Gln											384
15						gac Asp											432
20						ggc Gly 150											480
						tcc Ser											528
25						ctg Leu											576
	ggc	atg	gac	qaq	ctq	tac	aaq										597
30		_	_		_	Tyr	_								٠		
35	Gly	Met F2A	Asp 195	Glu	Leu M153		Lys	ions	+Met	- @ F	posit	. 1					
	Gly CFP SEQ atg	Met F2A ID 1	Asp 195 ,N14 NOS:9	Glu 16I/ 94 & ctg Leu	M153	Tyr	Lys utati	ttc	atc	tgc	acc	acc		_	_		48
35	CFP SEQ atg Met 1	F2A ID 1 ggc Gly	Asp 195 ,N14 NOS:9 aag Lys	Glu 16I/ 94 & ctg Leu	M153 95 acc Thr 5	Tyr 3T mu	Lys atati aag Lys gtg	ttc Phe acc	atc Ile acc	tgc Cys 10	acc Thr	acc Thr	Gly	Lys	Leu 15 cag	Pro tgc	48
35	Gly  CFP SEQ  atg Met 1  gtg Val	F2A ID 1 ggc Gly ccc Pro	Asp 195 ,N14 NOS:9 aag Lys tgg Trp	Glu 16I/ 94 & ctg Leu ccc Pro 20 tac	M153 95 acc Thr 5 acc Thr	Tyr  ST mu  ctg  Leu  ctc	Lys aag Lys gtg Val	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	acc Thr tac Tyr	Gly ggc Gly ttc	Lys ctg Leu 30	Leu 15 cag Gln aag	tgc Cys	
35	CFP SEQ atg Met 1 gtg Val ttc Phe	F2A ID N ggc Gly ccc Pro gcc Ala	Asp 195 ,N14 NOS:S aag Lys tgg Trp cgc Arg 35	Glu 46I/ 94 & ctg Leu ccc Pro 20 tac Tyr	M153 95 acc Thr 5 acc Thr	Tyr  Ctg Leu  Ctc Leu  gac	Lys  aag Lys  gtg Val  cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	acc Thr tac Tyr gac Asp	Gly ggc Gly ttc Phe 45	Lys ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	Pro tgc Cys tcc Ser	96

					gag Glu											288
5					aag Lys											336
10					aag Lys											384
15					gag Glu											432
20					atc Ile 150											480
		_	_		cag Gln		_	_	_		_			-	-	528
25					ctg Leu											576
30					ctg Leu											600
35			1L/S6 96 &		466W,	/N146	5 <b>I/M</b> 1	153T	muta	ation	ns		. •			
40	 _	_		_	aag Lys			_				_	_			48
45					gtg Val											96
,0	Arg				cac His											144
50					gtc Val											192
55					cgc Arg 70										ctg Leu 80	240

			ctg Leu											288
5			ctg Leu											336
10			cag Gln											384
15			gac Asp											432
20			ggc Gly 150											480
			tcc Ser											528
25			ctg Leu											576
30			tac Tyr											597
35		54L/8 98 &	/Y66W	N/N14	16I/N	M1537	r mut	catio	ons -	⊦ Met	: @ <u>r</u>	os.	1	
40			ctg Leu											48
45			ctc Leu											96
			gac Asp											144
50			tac Tyr											192
55			acc Thr 70											240

			cgc Arg										288
5			999 Gly 100			-				_		_	336
10			gcc Ala										384
15		_	aac Asn		_		_	_	_	_	-		432
20			acc Thr										480
20			agc Ser										528
25			atg Met 180										576
30			gac Asp										600
35			53A n										
40			acc Thr										48
45			acc Thr 20										96
50	gcc Ala		ccc Pro										144
50			ggc Gly										192
55			aag Lys										240

5						ctg Leu											288
						ctg Leu											336
10						cag Gln	_				_	_			_		384
15						gac Asp		_		_		_	_			_	432
20						ggc Gly 150											480.
25						tcc Ser											528
						ctg Leu											576
30						tac Tyr	_		-							·	597
35		F2A,				cion 3	+ M6	et @	posi	it. 1	L						
40						ctg Leu											48
45						ctc Leu											96
		_	_			gac Asp		_	_			_			_		144
50						tac Tyr											192
55	qac	ggc	aac	tac	aag	acc	cgc	gcc	gag Glu	gtg	aag	ttc	gag	ggc	gac	acc	240

			gag Glu								288
5			aag Lys								336
10			aag Lys								384
15	_		gag Glu	_		_	 -	_	-		432
20			atc Ile 150								480
			cag Gln								528
25			ctg Leu								576
30			ctg Leu								600
35		16I/ LO4 8	BA Mu 5	ıtati	ions						
40			aag Lys								48
45			gtg Val								96
			cac His								144
50			gtc Val								192
55			cgc Arg 70								240

						ctg Leu												288
5		_			_	ctg Leu						_			_			336
10		_	_	_	_	cag Gln	_					_			_			384
15						gac Asp											4	132
20						ggc Gly 150											4	180
		_	_		_	tcc Ser	_	_	_		_				_	_	5	528
25						ctg Leu											5	576
30						tac Tyr											5	597
35		F2A ID 1				3 <b>A</b> mi 7	itati	ions	+ M6	et @	posi	it. 1	L					
40	_		_	_		ctg Leu	_			_								48
	gtg	CCC			200	ata	~+~							a+~	cad	tac		96
45						Leu					ggc							
45	Val ttc	Pro	Trp	Pro 20 tac	Thr		Val cac	Thr	Thr 25 aag	Phe cgg	Gly	Tyr gac	Gly ttc	Leu 30 ttc	Gln	Cys tcc	1	144
50	ttc Phe	gcc Ala	Trp cgc Arg 35 ccc	Pro 20 tac Tyr	Thr ccc Pro	Leu gac	Val cac His	Thr atg Met 40 cag	Thr 25 aag Lys gag	Phe cgg Arg	Gly cac His	Tyr gac Asp	ttc Phe 45	Leu 30 ttc Phe	Gln aag Lys aag	tcc Ser		

						gag Glu												288
5						aag Lys							_			-		336
10						aag Lys												384
15						gag Glu												432
20		Gln				atc Ile 150											,	480
						cag Gln												528
25						ctg Leu												576
30						ctg Leu												600
35		F2A,				3 <b>A</b> mi 9	ıtati	ions			•							
40	Gly	Lys	Leu	Thr	Leu	aag Lys	Phe	Ile	Cys	Thr	Thr	Gly	Lys	Leu	Pro			48
45						gtg Val												.96
						cac His												144
50						gtc Val												192
55						cgc									acc Thr			240

					ctg Leu											288
5					ctg Leu											336
10					cag Gln											384
15					gac Asp											432
20					ggc Gly 150											480
					tcc Ser											528
25					ctg Leu											576
30 -				_	tac Tyr	_										597
35			53T/ 110 8		3 <b>A</b> mi 1	ıtat	ions	+ Me	et @	posi	it. 1	L				
40	Gly		Leu	Thr	ctg Leu	Lys	Phe	Ile	Cys	Thr						48
45					ctc Leu											96
					gac Asp											144
50					tac Tyr											192
	~~~	222	t > 0	224	200	cac	acc	gag	ata	aad	ttc	gag	aac	gac	acc	240

									gac Asp				288
5									tac Tyr				336
10			Ala						atc Ile				384
15									cag Gln				432
20									gtg Val 155				480
		_	_	_		_	_	_	aaa Lys	_		 _	528
25									acc Thr				576
30		_		 ctg Leu		_							600
35			46I/N 112 8	r/ V: 3	163A	muta	ation	ns					
40									acc Thr				48
45									tac Tyr				96
	Arg				Met				gac Asp				144
50									atc Ile				192
55									ttc Phe 75				240

						ctg Leu											288
5						ctg Leu											336
10						cag Gln											384
15						gac Asp											432
20						ggc Gly 150											480
						tcc Ser											528
25						ctg Leu											576
30						tac Tyr			٠								597
35			, N14 NOS:			r/ V1 5	163A	muta	ation	ns +	Met	@ pc	s.1				
40		Gly		Leu	Thr	ctg Leu	Lys	Phe	Ile	Cys	Thr			Lys			48
										10					-0		
45				CCC	acc	ctc Leu	gtg	acc	acc	ttc	ggc			ctg	cag		96
45	Val ttc	Pro gcc	Trp	ccc Pro 20	acc Thr	ctc	gtg Val cac	acc Thr	acc Thr 25 aag	ttc Phe cgg	ggc Gly cac	Tyr gac	Gly	ctg Leu 30	cag Gln aag	Cys	96
45 50	ttc Phe gcc	Pro gcc Ala atg	Trp cgc Arg 35 ccc	ccc Pro 20 tac Tyr	acc Thr ccc Pro	ctc Leu gac	gtg Val cac His	acc Thr atg Met 40 cag	acc Thr 25 aag Lys	ttc Phe cgg Arg	ggc Gly cac His	Tyr gac Asp atc	ttc Phe 45	ctg Leu 30 ttc Phe	cag Gln aag Lys	Cys tcc Ser	

						gag Glu												288
5						aag Lys												336
10						aag Lys												384
15						gag Glu												432
20		Gln				atc Ile 150												480
						cag Gln												528
25						ctg Leu												576
30						ctg Leu												600
35			A , F4 NOS: 3			/Y66V 7	N/N14	16I/N	11537	Γ/V16	53A/I	[203 <u>]</u>	/ mut	atio	ons			
40						aag Lys												48
45						gtg Val			_				_	_	_			96
50						cac His												144
	Met	Pro 50	Glu	Gly	Tyr	gtc Val	Gln 55	Glu	Arg	Thr	Ile	Phe 60	Phe	Lys	Āsp		• • •	192
55						gcc Arg 70										Leu 80	240	

					ctg Leu												288
5		-		_	ctg Leu						_			_			336
10					cag Gln												384
15					gac Asp		_		_		_	_			_		432
20					ggc Gly 150												480
					tcc Ser										cgc Arg		528
25					ctg Leu												576
30					tac Tyr												597
35	pos	. 1	16L/8 118 8			N/N14	16I/N	41537	Γ/V1 <i>6</i>	53A/I	r2033	/ mut	atio	ons 4	⊦ Met	@	
40					ctg Leu												48
45					ctc Leu				_				_	_	_		96
.0					gac Asp												144
50					tac Tyr												192
55					acc Thr 70												240

	_			_		gag Glu	_	_		_			 _		288
5						aag Lys									336
10						aag Lys									384
15						gag Glu									432
20						atc Ile 150									480
						cag Gln									528
25	_	-		_	_	ctg Leu	_				_	_			576
30			_	_		ctg Leu		_							600
35				146I, 120 8		3Y mi 1	ıtat:	ions							
40						aag Lys									48
45						gtg Val									96
						cac His	Met								144
50						gtc Val									192
55						cgc Arg 70									240

						ctg Leu											288	
5						ctg Leu						_			_		336	
10						cag Gln											384	
15						gac Asp											432	
20						ggc Gly 150											480	
		_	_		_	tcc Ser	-	_	_		_				_	_	528	
25						ctg Leu											576	
20						tac											597	
30	GIY	Met	<b>Asp</b> 195	Glu	Leu	Tyr	Lys								·			
35	CGFI	? F2 <i>I</i>	195 A, N		<sup>/</sup> T203	 3Y mu	_	ions	+ Me	et @	posi	lt. 1	L					
	CGFI SEQ atg	F21 ID 1	195 A, Ni NOS:	146I, 122 8 ctg	'T203 2 123 acc	 3Y mu	ıtati	ttc	atc	tgc	acc	acc	ggc				48	
35	CGFI SEQ atg Met 1	P F2A ID N ggc Gly ccc	195 A, Ni NOS: aag Lys	t46I, t22 8 ctg Leu ccc	/T203 acc Thr 5	3Y mu 3 ctg	aag Lys gtg	ttc Phe acc	atc Ile acc	tgc Cys 10	acc Thr	acc Thr	ggc Gly	Lys	Leu 15 cag	Pro tgc	48 96	
35	CGFI SEQ atg Met 1 gtg Val	P F21 ID 1 ggc Gly ccc Pro	195 A, N: NOS: aag Lys tgg Trp	ctg Leu ccc Pro 20	T203 acc Thr acc Thr ccc	3Y mu 3 ctg Leu ctc	aag Lys gtg Val	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	acc Thr tac Tyr	ggc Gly ggc Gly	Lys ctg Leu 30	Leu 15 cag Gln aag	Pro tgc Cys		
35	CGFF SEQ atg Met 1 gtg Val ttc Phe	ggc Gly ccc Pro gcc Ala	195 A, N: NOS: aag Lys tgg Trp cgc Arg 35	ctg Leu ccc Pro 20 tac Tyr	ACC Thr 5 acc Thr CCC Pro	3Y mm 3 Ctg Leu Ctc Leu	aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	acc Thr tac Tyr gac Asp	ggc Gly ggc Gly ttc Phe 45	ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	tgc Cys tcc Ser	96	

					gag Glu									288
5					aag Lys									336
10					aag Lys									384
15					gag Glu									432
20					atc Ile 150									480
					cag Gln		_	_	_		-		 aag Lys	528
25					ctg Leu									576
30		_	_		ctg Leu		_							600
35			153T, 124 8		BY mu	ıtati	ions							
40			Thr		aag Lys									48
45					gtg Val									96
	_			_	cac His	_	_			_		_	_	144
50					gtc Val									192
55					cgc Arg 70									240

					ctg Leu										288
5					ctg Leu										336
10		_	Asp	_	cag Gln	_				_			_		384
15					gac Asp										432
20					ggc Gly 150										480
					tcc Ser										528
25		_	_	_	ctg Leu					_	_				576
30			_	_	tac Tyr	_					•				597
35			153T/ 126 8		3Υ mι 7	ıtat	ions	+ M6	et @	posi	it. 1	L			
40			Leu		ctg Leu										48
45					ctc Leu										96
	Ala				gac Asp										144
50					tac Tyr										192
55					acc Thr 70										240

											gag Glu		288
5											cac His 110		336
10											aac Asn		384
15											gac Asp		432
20											ccc Pro		480
											aac Asn		528
25											999 Gly 190		576
30			_	_	 ctg Leu		_						600
35				16I/ 128 8	3T/T2 9	203Y	muta	ation	ns				
40											ctg Leu		48
45											cag Gln 30		96
											aag Lys		144
50											aag Lys		192
55	ggc	aac						gtg Val			gac		240

						ctg Leu											288
5						ctg Leu											336
10				Asp		cag Gln											384
15						gac Asp											432
20		Asn				ggc Gly 150											480
						tcc Ser										cgc Arg	528
25						ctg Leu											576
30						tac Tyr											597
35		9 F2 <i>I</i> ID N				BT/T2 L	203Y	muta	ation	ıs +	Met	@ pc	osit.	. 1			
35 40	SEQ atg	ID N	NOS:1	ctg Leu	acc		aag	ttc	atc	tgc	acc	acc	ggc	aag			48
40	sEQ atg Met 1 gtg	ccc Gly ggc	aag Lys tgg	ctg Leu ccc	acc Thr 5	l ctg	aag Lys gtg	ttc Phe	atc Ile acc	tgc Cys 10 ttc	acc Thr	acc Thr	ggc Gly	aag Lys ctg	Leu 15 cag	Pro tgc	48
	atg Met 1 gtg Val	ggc Gly ccc Pro	aag Lys tgg Trp	ctg Leu ccc Pro 20	acc Thr 5 acc Thr	ctg Leu ctc	aag Lys gtg Val	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	acc Thr tac Tyr	ggc Gly ggc Gly	aag Lys ctg Leu 30	Leu 15 cag Gln aag	Pro tgc Cys	
40	sEQ atg Met 1 gtg Val ttc Phe	ggc Gly ccc Pro	aag Lys tgg Trp cgc Arg 35	ctg Leu ccc Pro 20 tac Tyr	acc Thr 5 acc Thr ccc Pro	ctg Leu ctc Leu	aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	acc Thr tac Tyr gac Asp	ggc Gly ggc Gly ttc Phe 45	aag Lys ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	tgc Cys tcc Ser	96

	_			_		gag Glu	_	_			_		_		_		288
5						aag Lys											336
10						aag Lys											384
15						gag Glu											432
20						atc Ile 150											480
						cag Gln											528
25						ctg Leu											576
30						ctg Leu										·	600
35		P F2 <i>I</i> ID 1				Г/Y66 В	SW/N1	L46I,	/M153	3T/T2	203Y	muta	ation	ıs			
40			Leu		Leu	aag Lys			Cys								48
45						gtg Val											96
						cac His											144
50						gtc Val											192
55						cgc Arg 70											240

					ctg Leu												288
5					ctg Leu												336
10					cag Gln												384
15					gac Asp												432
20					ggc Gly 150												480
					tcc Ser												528
25					ctg Leu												576
30					tac Tyr												597
35			L/S6			/N146	5I/M1	L53T/	/T203	3Υ mι	ıtati	ions	+ Me	et @	pos.	1	
40		Lys		Thr	ctg Leu				Cys								48
45					ctc Leu												.96
					gac Asp	His											144
50					tac Tyr												192
55					acc Thr 70												240

			gag Glu								288
5			aag Lys								336
10			aag Lys								384
15			gag Glu							•	432
20			atc Ile 150								480
			cag Gln						aag Lys	•	528
25			ctg Leu								576
30			ctg Leu								600
35		L63A/ L36 &	3Y mu 7	ıtati	ions						
40			aag Lys								48
45			gtg Val								96
			cac His								144
50			gtc Val								192
55			cgc Arg 70								240

				ctg Leu										288
5				ctg Leu										336
10			_	cag Gln	_				_	_		_		384
15				gac Asp										432
20				ggc Gly 150										480
				tcc Ser									cgc Arg	528
25				ctg Leu										576
30				tac Tyr										597
35		A, NI NOS:1		BA/T2 L	203Y	muta	ation	ns						
40		Leu	Leu	aag Lys			Cys							48
45				gtg Val										96
			_	cac His	_	_			_		_		_	144
50				gtc Val										192
55				cgc Arg 70										240

						ctg Leu											288
5						ctg Leu											336
10						cag Gln											384
15						gac Asp											432
20						ggc Gly 150											480
						tcc Ser											528
25	_		_	_	_	ctg Leu					_	_					576
30						tac Tyr											597
35			A. N.	1461,	/V163	BA/T2	203Y	muta	at i or	1G T		_					
	OD <sub>Q</sub>	ID 1			x 143	3				15 +	Met	@ pc	os. I	L			
40	atg	ggc	NOS: aag Lys	142 8 ctg Leu	acc	ctg Leu		ttc	atc Ile	tgc	acc	acc	ggc	aag			48
	atg Met 1 gtg	ggc Gly ccc	aag Lys tgg	ctg Leu ccc	acc Thr 5	ctg Leu	Lys gtg	ttc Phe	atc Ile acc	tgc Cys 10	acc Thr	acc Thr	ggc Gly	aag Lys ctg	Leu 15 cag	Pro tgc	<b>48</b> 96
40 45	atg Met 1 gtg Val	ggc Gly ccc Pro	aag Lys tgg Trp	ctg Leu ccc Pro 20	acc Thr 5 acc Thr	ctg Leu ctc	Lys gtg Val cac	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	acc Thr tac Tyr	ggc Gly ggc Gly	aag Lys ctg Leu 30	Leu 15 cag Gln aag	Pro tgc Cys	
	atg Met 1 gtg Val ttc Phe	ggc Gly ccc Pro gcc Ala	aag Lys tgg Trp cgc Arg 35	ctg Leu ccc Pro 20 tac Tyr	acc Thr 5 acc Thr ccc Pro	ctg Leu ctc Leu	Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	acc Thr tac Tyr gac Asp	ggc Gly ggc Gly ttc Phe 45	aag Lys ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	tgc Cys tcc Ser	96

						gag Glu											288
5						aag Lys											336
10						aag Lys											384
15		_				gag Glu	_		_		_		_	_			432
20						atc Ile 150											480
			_	_		cag Gln		_	_	_		_				_	528
25						ctg Leu											576
30						ctg Leu											600
35				153T, 144 8		3 <b>A/T</b> 2	203Y	muta	ation	າຣ							
35 40	SEQ ggc	ID Naag	ios::	144 8 acc	2 145 ctg		ttc	atc	tgc	acc							48
	ggc Gly 1	ID Naag Lys	ctg Leu ccc	acc Thr	ctg Leu 5	aag	ttc Phe	atc Ile acc	tgc Cys ttc	acc Thr 10	Thr	Gly	Lys	Leu	Pro 15 tgc	Val	96
40	ggc Gly 1 ccc Pro	aag Lys tgg Trp cgc	ctg Leu ccc Pro	acc Thr acc Thr 20	ctg Leu 5 ctc Leu gac	aag Lys gtg	ttc Phe acc Thr	atc Ile acc Thr	tgc Cys ttc Phe 25	acc Thr 10 ggc Gly	Thr tac Tyr	Gly ggc Gly ttc	Lys ctg Leu	Leu cag Gln 30	Pro 15 tgc Cys	Val ttc Phe	
40	ggc Gly 1 ccc Pro gcc Ala	aag Lys tgg Trp cgc Arg	ctg Leu ccc Pro tac Tyr 35	acc Thr acc Thr 20 ccc Pro	ctg Leu 5 ctc Leu gac Asp	aag Lys gtg Val	ttc Phe acc Thr atg Met	atc Ile acc Thr aag Lys 40	tgc Cys ttc Phe 25 cgg Arg	acc Thr 10 ggc Gly cac His	tac Tyr gac Asp	Gly  ggc Gly  ttc Phe	Lys  ctg Leu  ttc Phe 45	cag Gln 30 aag Lys	Pro 15 tgc Cys tcc ser	Val ttc Phe gcc Ala	96

						ctg Leu											288
5						ctg Leu										tat Tyr	336
10						cag Gln											384
15						gac Asp											432
20						ggc Gly 150											480
						tcc Ser											528
25						ctg Leu											576
30 -						tac Tyr											597
35		P F27				3A/T2 7	203Y	muta	ation	1S +	Met	@ pc	os. 1	L	•		
40						ctg Leu											48
45						ctc Leu								_	_	_	96
45	Val ttc	Pro gcc	Trp	Pro 20 tac	Thr		Val	Thr atg	Thr 25 aag	Phe cgg	Gly	Tyr gac	Gly	Leu 30	Gln	Cys	96 144
50	ttc Phe	Pro gcc Ala atg	Trp cgc Arg 35	Pro 20 tac Tyr	Thr ccc Pro	Leu gac	Val cac His	Thr atg Met 40 cag	Thr 25 aag Lys gag	Phe cgg Arg	Gly cac His	Tyr gac Asp atc	Gly ttc Phe 45	Leu 30 ttc Phe	Gln aag Lys aag	Cys tcc Ser	

			gag Glu									288
5			aag Lys									336
10			aag Lys									384
15	_		gag Glu	_		_		_	_	_		432
20			atc Ile 150									480
			cag Gln									528
25			ctg Leu									576
30			ctg Leu									600
35		146I, 148 8	BT/V1 9	L63A,	/T203	BY mu	utati	lons				
40			aag Lys									48
45			gtg Val									96
	Arg		cac His									144
50			gtc Val									192
55			cgc Arg 70									240

				ctg Leu											288
5			-	ctg Leu						_			_		336
10				cag Gln											384
15				gac Asp											432
20				ggc Gly 150											480
				tcc Ser											528
25				ctg Leu											576
30				tac Tyr								,			597
35		A, N14 NOS:		r/V16 L	3A/1	[203 <u>]</u>	' mut	atio	ons 4	⊦ Met	: @ <u>r</u>	oosit	:: 1		
40		Lys	Thr	ctg Leu				Cys							48
45				ctc Leu											96
				gac Asp											144
50				tac Tyr											192
55				acc Thr 70											240

									gac Asp					288
5									tac Tyr					336
10			_	_	_	_	_		 atc Ile	_	_		_	384
15									cag Gln					432
20	Gln								gtg Val 155					480
									aaa Lys				aag Lys	528
25									acc Thr					576
30					ctg Leu									600
35			5H mi L52 8											
40		Leu		Leu				Cys	acc Thr					48
45									cac His					96
	Arg								gac Asp					144
50									atc Ile					192
55									ttc Phe 75					240

					aag Lys							288
5					gag Glu							336
10					aag Lys							384
15					ggc Gly 135							432
20					gac Asp							480
					gcc Ala							528
25					gag Glu							576
30				tac Tyr								597
35	F2A				+ M6	et @	posi	it. :	L			
40		Lys	Thr	Leu	aag Lys	Phe		Cys				48
45					gtg Val							96
					cac His							144
50					gtc Val 55							192
55					cgc Arg							240

						gag Glu									288
5						aag Lys									336
10						aag Lys									384
15						gag Glu									432
20						atc Ile 150									480
20						cag Gln									528
25						ctg Leu									576
30						ctg Leu									600
35				15F n											
40	Gly	Lys	Leu	Thr	Leu	aag Lys	Phe	Ile	Cys	Thr	Thr	Lys	Pro		48
45						gtg Val									.96
						cac His									144
50						gtc Val									192 <sup>.</sup>
55						cgc Arg 70								· ·	240

					ctg Leu										288
5				_	ctg Leu						_		-		336
10					cag Gln										384
15					gac Asp										432
20					ggc Gly 150										480
					tcc Ser										528
25					ctg Leu										576
30					tac Tyr										597
35			15F r 158 8		ion	+ Me	et @	posi	ition	n 1					
40		Lys		Thr	ctg Leu			Ile							48
45					ctc Leu										96
					gac Asp										144
50					tac Tyr										192
55					acc Thr										240

				gag Glu									288
5		_		aag Lys	_						_		336
10				aag Lys									384
15				gag Glu									432
20				atc Ile 150									480
				cag Gln							aag Lys		528
25				ctg Leu								·	576
30				ctg Leu									600
35	F2A,			muta L	ation	ıs							
40			Leu	aag Lys			Cys						48
45				gtg Val									96
				cac His									144
50				gtc Val									192
55				cgc Arg 70									240

						ctg Leu											288
5		_				ctg Leu						_			-		336
10						cag Gln											384
15	-					gac Asp		_		_		_	_			_	432
20						ggc Gly 150											480
						tcc Ser											528
25						ctg Leu											576
	ggc	atg	gac	gag	ctg	tac	aag										597
30	Gly	Met	Asp 195		Leu	Tyr	Lys										
35	BFP	F2A,	195	Glu SH/Y:	L45F	muta		1S +	Met	@ pc	os. 1	L					
	BFP SEQ atg	F2A, ID 1	195 , Y66 NOS::	Glu 5H/Y: L62 & ctg Leu	l45F ≩ 163 acc	muta	ation aag	ttc	atc	tgc	acc	acc					48
35	BFP SEQ atg Met 1	F2A, ID 1 ggc Gly	195 , Y66 NOS:1 aag Lys	Glu 5H/Y: 162 & ctg Leu ccc	acc Thr acc	muta 3 ctg	ation aag Lys gtg	ttc Phe	atc Ile acc	tgc Cys 10	acc Thr	acc Thr	Gly	Lys	Leu 15 cag	Pro tgc	48
35	BFP SEQ atg Met 1 gtg Val	F2A, ID 1 ggc Gly ccc Pro	195 , Y66 NOS:: aag Lys tgg Trp	Glu  GH/Y: L62 &  ctg Leu  ccc Pro 20  tac	acc Thr 5 acc Thr	muta 3 ctg Leu ctc	ation aag Lys gtg Val cac	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	acc Thr cac His	Gly ggc Gly ttc	Lys ctg Leu 30	Leu 15 cag Gln aag	Pro tgc Cys	
35	BFP SEQ atg Met 1 gtg Val ttc Phe	F2A, ID N ggc Gly ccc Pro gcc Ala	195 , Y66 NOS: aag Lys tgg Trp cgc Arg 35 ccc	Glu  SH/Y: 162 8  ctg Leu  ccc Pro 20  tac Tyr	acc Thr 5 acc Thr ccc Pro	muta } ctg Leu ctc Leu	ation aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	acc Thr cac His gac Asp	Gly ggc Gly ttc Phe 45	ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	Pro tgc Cys tcc Ser	96

				gag Glu										288
5				aag Lys	_					_			-	336
10				aag Lys										384
15				gag Glu										432
20				atc Ile 150										480
		_	_	cag Gln		_	_	_	_				_	528
25				ctg Leu										576
30				ctg Leu										600
35			1L/Y6 164 8	/145E	7 mut	atio	ons				_			
40				aag Lys										48
45				gtg Val										96
	Arg			cac His										144
50		-		gtc Val	_		-				_	_	_	192
55				cgc Arg 70										240

					ctg Leu											288
5					ctg Leu											336
10		_	_	_	cag Gln	_				_			_		·	384
15					gac Asp											432
20					ggc Gly 150											480
					tcc Ser									cgc Arg		528
25					ctg Leu											576
30					tac Tyr											597
35		, F64 NOS:1			Y145E 7	7 mut	atio	ons -	+ Met	: @ r	posit	. 1				
40					ctg Leu											48
45					ctc Leu											96
					gac Asp											144
50					tac Tyr											192
55					acc Thr 70											240

									gac Asp				288
5		_			_	_			ttc Phe	_		_	336
10									atc Ile				384
15									cag Gln				432
20									gtg Val 155				480
									aaa Lys				528
25									acc Thr				576
30		_	-		ctg Leu		_						600
35			Met 168 &	_	osit. 9	. 1					٠		
40			Leu						acc Thr				48
45									acc Thr				96
	Ser					His			cac His				144
50									acc Thr				192
55									aag Lys 75				240

			cgc Arg												288
5			999 Gly 100		_	_						_		_	336
10			gcc Ala												384
15			aac Asn		Glu										432
20			acc Thr												480
		_	agc Ser		_		_	_	_		_			 _	528
25			atg Met 180												576
30		_	gac Asp		_		_								600
35			d F2 <i>I</i> 170 8			572A,	/N149	K/M1	L53T/	/1167	7T mu	ıtati	ions		
40			acc Thr	_	_			_				_	_		48
45			acc Thr 20												96
70			ccc Pro												144
50			ggc Gly												192

								ggc Gly										288
5		_			_	_		tac Tyr				_		_	_			336
10								aac Asn 120										384
15								agc Ser										432
20								ggc Gly										480
								ctg Leu										528
25								ttc Phe										576
30						tac Tyr												597
35	EGF1	eme	eralo	d F2 <i>I</i>	A. Se	55T/S	572 <b>A</b> /	/N149	ek/Mi	153T/	/I167	7T mi	ıtat:	ions	+ Me	et @ p	os.	1
	SEQ	ID 1	10S:	L72 8	x 173	3										-		
40				Leu				ttc Phe	Ile									48
45								acc Thr						_	_	_		96
								atg Met 40										144
			35															
50	gcc		ccc					cag Gln										192

				gag Glu								288
5				aag Lys						gtc Val	•	336
10				aag Lys								384
15				gag Glu								432
20				atc Ile 150							•	480
				cag Gln							•	528
25				ctg Leu							· !	576
30				ctg Leu						٠.	,	600
35	F2A, ID 1											
40			Leu	aag Lys		Cys						48
45				gtg Val								96
				cac His							:	144
50				gtc Val								192
55				cgc Arg 70							:	240

											gac Asp			288
5											aac Asn 110			336
10											ttc Phe			384
15											cac His			432
20	Asn										gac Asp			480
											gag Glu			528
25											atc Ile 190			576
30				tac Tyr		•				•				597
35		, Y20 NOS:1			+ Me	et @	pos.	. 1						
40											aag Lys			48
45											ctg Leu 30			96
				_		_	_		_		ttc Phe	_		144
50											ttc Phe			192
55											ggc Gly			240

								atc Ile 90						288
5				_	_	_		aac Asn				_	3	336
10								ggc Gly					3	884
15								gtg Val					4	132
20								ccc Pro					4	180
		_	_	_		_	_	agc Ser 170		_		 aag Lys	5	528
25								gtg Val					5	576
30			gac Asp										6	500
35			03Η π 178 8						•					
40								acc Thr 10						48
45								ggc Gly						96
								cac His					1	.44
50								acc Thr					. 1	.92
55								aag Lys					2	240

									gac Asp 90						2	88
5									tac Tyr							36
10									atc Ile						3	84
15	_			_	_		_		cag Gln		_	_		_	4	32
20									gtg Val						4	80
									aaa Lys 170						5	28
25	_	_	_	_	_				acc Thr	_	_				5	76
30					tac Tyr										5	97
35			03H n			+ M€	et @	posi	it. 1	L						
40									tgc Cys 10							48
45									ttc Phe							96
50									cgg Arg						1	44
									cgc Arg						1	92
55									gtg Val						2	40

5							aag Lys									288
							gag Glu									336
10							aag Lys 120									384
15							ggc Gly									432
20							gac Asp									480
25							gcc Ala								aag Lys	528
							gag Glu									576
30					ctg Leu											600
35				rag 182 8		39 oi	E SI	EYFP)	+ 1	Met @	) pos	s. 1				
40	_		_	_	_	_	ttc Phe		_				_	_		48
45							acc Thr									96
		Ala					atg Met 40	_			_			-		144
50		Met					cag Gln									192
		50				55					00					

					gag Glu									288
5					aag Lys									336
10					aag Lys									384
15					gag Glu							tac Tyr	<b>J</b>	432
20					atc Ile 150									480
					cag Gln									528
25					ctg Leu									576
30 -					ctg Leu					·	·			600
35	ısF2 <i>I</i> ID N	•	51750 L84 8		atio 5	on								
40		Leu		Leu	aag Lys		Cys							48
45	_				gtg Val				 _	_	_			96
					cac His									144
50					gtc Val									192
55					cgc Arg 70									240

						ctg Leu												288
5						ctg Leu												336
10						cag Gln												384
15						gac Asp												432
20		Asn				ggc Gly 150											,	480
						tcc Ser												528
25						ctg Leu											,	576
30						tac Tyr	_	·									:	597
35			A. S	:1750	3 mut	atio	n +	Mot										
		ID N	NOS:			7		ricc	⊕ pc	osit.	. 1							
40		ggc	NOS:1	.86 8 ctg Leu	2 187 acc	7 ctg Leu	aag	ttc	atc	tgc	acc							48
	Met 1 gtg	ggc Gly	aag Lys tgg	ctg Leu ccc	acc Thr 5	ctg	aag Lys gtg	ttc Phe	atc Ile	tgc Cys 10	acc Thr	Thr tac	Gly	Lys	Leu 15 cag	Pro tgc		48
45	Met 1 gtg Val	ggc Gly ccc Pro	aag Lys tgg Trp	ctg Leu ccc Pro 20	acc Thr 5 acc Thr	ctg Leu ctc	aag Lys gtg Val	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr ggc Gly	Thr tac Tyr gac	Gly ggc Gly ttc	Lys ctg Leu 30	Leu 15 cag Gln	tgc Cys		
	Met 1 gtg Val ttc Phe gcc	ggc Gly ccc Pro gcc Ala	aag Lys tgg Trp cgc Arg 35	ctg Leu ccc Pro 20 tac Tyr	acc Thr 5 acc Thr ccc Pro	ctg Leu ctc Leu	aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	tac Tyr gac Asp	Gly  ggc Gly  ttc Phe 45	Ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	tgc Cys tcc Ser		96

				gag Glu									288
5				aag Lys									336
10				aag Lys									384
15				gag Glu									432
20				atc Ile 150									480
		_	_	cag Gln		_	_	_	_			_	528
25				ctg Leu									576
30				ctg Leu									600
35	usF2/ ID 1			L75G 9	muta	ation	ns						
40				aag Lys									48
45				gtg Val									96
10				cac His									144
50				gtc Val	_		_			_	_	_	192
55				cgc Arg 70									240

											ttc Phe						288
5											aac Asn						336
10											aag Lys						384
15											ctc Leu						432
20											ctg Leu 155						480
											gac Asp						528
25											gcc Ala						576
30			gac Asp 195														597
35		ısF2 <i>I</i>	A, N NOS:1				muta	ation	1S +	Met	@ pc	osit.	. 1				
40	atg	ggc	aag	ctg Leu	acc	ctg Leu	Lys	Phe	Ile	Cys	acc Thr	Thr	Gly	Lys	Leu		48
45											ggc Gly						96
		Ala					His				cac His						144
50											acc Thr						192 <sup>-</sup>
55											aag Lys 75						240

			gag Glu								288
5			aag Lys								336
10			aag Lys								384
15			gag Glu								432
20			atc Ile 150								480
			cag Gln								528
25			ctg Leu								576
30			ctg Leu								600
35		/163 <i>I</i> 192 8	L75G 3	muta	ation	ns					
40		Thr	aag Lys								48
45			gtg Val								96
			cac His	_	_		_		_	_	144
50			gtc Val								192
55			cgc Arg 70								240

											ttc Phe						288
5											aac Asn						336
10											aag Lys						384
15											ctc Leu						432
20											ctg Leu 155						480
											gac Asp					cgc Arg	528
25	_		_	_	_	_					gcc Ala	_					576
			gac		ctg												597
30	Gly	Met	Asp 195	Glu	Leu	Tyr	Lys		,								
35	Ven	ısF2/	195	/163 <i>l</i>	A, S:	175G	_	ation	ns +	Met	@ pc	os. 1	L	<i>.</i>			
	Venu SEQ atg	ısF2 ID 1 ggc Gly	195 A, NOS:	/163/ 194 8 ctg Leu	A, S: 2 195 acc Thr	175G 5 ctg Leu	muta aag Lys	ttc Phe	atc Ile	tgc Cys	@ po acc Thr	acc Thr	ggc Gly	Lys	Leu		48
35 40	Venu SEQ atg Met 1	isF2i ID i ggc Gly ccc	195 A, NOS:	/163/ 194 { ctg Leu ccc	A, S: acc Thr 5	175G 5 ctg Leu	muta aag Lys gtg	ttc Phe	atc Ile acc	tgc Cys 10	acc Thr	acc Thr	ggc Gly	Lys	Leu 15 cag	Pro tgc	48
35	Venu SEQ atg Met 1 gtg Val	usF21 ID 1 ggc Gly ccc Pro gcc Ala	195 A, VNOS:: aag Lys tgg Trp	/163/ 194 & ctg Leu ccc Pro 20	A, S: acc Thr ccc	ctg Leu ctc Leu	muta aag Lys gtg Val cac	ttc Phe acc Thr	atc Ile acc Thr 25	tgc Cys 10 ttc Phe	acc Thr	acc Thr tac Tyr	ggc Gly ggc Gly	Lys ctg Leu 30	Leu 15 cag Gln aag	Pro tgc Cys	
35 40	Venu SEQ atg Met 1 gtg Val ttc Phe	usF22 ID 1 ggc Gly ccc Pro gcc Ala	195 A, NOS: aag Lys tgg Trp cgc Arg 35 ccc	Ctg Leu ccc Pro 20 tac Tyr	acc Thr 5 acc Thr ccc Pro	ctg Leu ctc Leu gac Asp	muta aag Lys gtg Val cac His	ttc Phe acc Thr atg Met 40 cag	atc Ile acc Thr 25 aag Lys	tgc Cys 10 ttc Phe cgg Arg	acc Thr ggc Gly cac His	acc Thr tac Tyr gac Asp	ggc Gly ggc Gly ttc Phe 45	ctg Leu 30 ttc Phe	Leu 15 cag Gln aag Lys	tgc Cys tcc Ser	96

						gag Glu											2	88
5						aag Lys												36
10						aag Lys											3	884
15						gag Glu											4	32
20						atc Ile 150											4	80
20						cag Gln		_	_	_		_				aag Lys	. 5	28
25						ctg Leu											5	76
30						ctg Leu			÷								6	00
35				16L/E 196 &		/M153 7	BT/VI	L63A,	/S175	5G mu	ıtati	ions						
40						aag Lys												48
45						gtg Val												96
50	gcc Ala	cgc Arg	tac Tyr 35	ccc Pro	gac Asp	cac His	atg Met	aag Lys 40	cgg Arg	cac His	gac Asp	ttc Phe	ttc Phe 45	aag Lys	tcc Ser	gcc Ala	1	44
55						gtc Val											1	92
55						cgc Arg 70											2	40

5					ctg Leu											288
				_	ctg Leu						_			_		336
10		_	_	_	cag Gln	_				_	_			_		384
15					gac Asp											432
20					ggc Gly 150											480
25					tcc Ser										cgc Arg	528
					ctg Leu											576
30				-	tac Tyr	_										597
35		A F4			/M153	BT/VI	L63 <b>A</b> /	/S175	5G mu	ıtati	ions	+ Me	et @	pos.	. 1	
40					ctg Leu	_	_		_				_	_		48
45					ctc Leu								_	_	_	96
					gac Asp	His										144
50					tac Tyr										gac Asp	192
55					acc Thr 70											240

	_			_		gag Glu	_	_			_		_		_		28	38
5						aag Lys											33	36
10						aag Lys											38	34
15						gag Glu											43	32
20						atc Ile 150											48	30
				_		cag Gln		_	-			_				aag Lys	52	28
25						ctg Leu											57	76
30 .						ctg Leu		_									60	
35				res: 200 8		3 1-1 1	L03 d	of YE	P)	pos	. 1 N	Met 1	remov	red				
40						gag Glu											4	8
45						gta Val											9	96
						acc Thr											14	14
50	Gly	Glu	Gly 35 ggc	Asp	Ala		Tyr gtg	Gly 40 ccc	Lys	Leu	Thr acc	Leu	Lys 45 gtg	Phe acc	Ile acc	Cys	14 19	

			ttc Phe												288
5			ttc Phe 100	_	_										306
10			L/66' 202 ≀			5									
15			aag Lys												48
20			gac Asp 20												96
25			ggc Gly										atc Ile		144
25			ggc Gly												192
30			ggc Gly												240
35	cgg		ttc Phe											:	288
40	_		ttc Phe 100	Phe	_	_									309
45			5L/66 204 8			BS p	os.1	. Met	ren	noved	ì .				
50			ggc Gly												48
50			ggc Gly 20												96
55			gat Asp												144

5							gtg Val 55									1	92
							ttc Phe									. 2	40
10							gcc Ala									2	88
15					aag Lys	-										3	06
20		F1B ID 1			utat: % 201												
25							gag Glu										48
	_		_	_		_	gta Val				_		_				96
30							acc Thr									1.	44
35							ccc Pro 55								acc Thr	1:	92
40							tgc Cys									24	40
45							tcc Ser									2	88
					ttc Phe											3	09
50	YFP SEQ				nutat 209		and	pos	s. 1	Met	remo	oved				·	
55							ctg Leu									•	48

					gta Val									96
5					acc Thr									144
10					ccc Pro									192
15			 -	_	tgc Cys 70		_	_		_	_	_		240
20					tcc Ser									288
20			ttc Phe 100	_	_									306
25		F1B ID N	5F mi 210 &		_									
30					gag Glu									48
35	gtc Val				gac Asp									96
40					gcc Ala									144
					ctg Leu									192
45					cag Gln 70									240
50					aag Lys									288
55	_				aag Lys	_								309

YFP F1B Y66F mutation and pos. 1 Metremoved SEQ ID NOS:212 & 213

5					gag Glu							48
10	 _	-		_	gta Val			_	_			 96
15					acc Thr							144
					ccc Pro							192
20			-	_	tgc Cys 70	_	_		_	_	-	 240
25					tcc Ser							288
30	atc Ile			_	_							306
35	F1B ID 1			ıtat: k 21								
40					gag Glu							48
45	 	_	_		gac Asp							 96
.0					gcc Ala							144
50					ctg Leu							192

												gtc Val		288
5	_		ttc Phe 100		_	_			•					309
10			9K mi 216 8			and p	pos.	L Met	cremo	oved				
15												atc Ile		48
20												tcc Ser 30		96
20												ttc Phe		144
25												acc Thr		192
30												atg Met		240
35												cag Gln		288
40			ttc Phe 100	_	_									306
45			9R mι 218 δ											
70												ccc Pro		48
50												gtg Val 30		96
55												aag Lys		144

					ctg Leu										192
5					cag Gln 70										240
10					aag Lys										288
15	_				aag Lys									•	309
		٠													
20		F1B ID N		utat: § 22:	ion a	and p	pos.	·1 M€	et re	emove	ed				
25					gag Glu										48
					gta Val										· 96
30					acc Thr			_	_		-	_		_	144
35					ccc Pro										192
40					tgc Cys 70										240
45					tcc Ser										288
40				aag Lys	-										306
50				/68L,	Q6 <u>9</u>	9Μ mι	ıtati	lons							
55					gag Glu										48

						gac Asp											96
5						gcc Ala											144
10						ctg Leu											192
15						atg Met 70											240
20						aag Lys											288
20						aag Lys											309
25			F1B, NOS:2		/68L,	, Q69 5	∂M mı	utati	ions	and	pos.	.1 M∈	et re	emove	ed		
30						gag Glu											48
																	0.5
35				ggc Gly 20		gta Val											96
<b>35 40</b>	ggc	Leu gag	Asp ggc	Gly 20 gat	Asp		Asn tac	ggc	His 25 aag	Lys ctg	Phe acc	Ser	Val	Ser 30 ttc	Gly	Glu tgc	144
35 40	ggc Gly acc	Leu gag Glu acc	Asp ggc Gly 35	Gly 20 gat Asp	Asp gcc Ala ctg	Val acc	Asn tac Tyr	Gly ggc Gly 40 ccc	His 25 aag Lys tgg	Lys ctg Leu ccc	Phe acc	ser ctg Leu	Val aag Lys 45	Ser 30 ttc Phe	Gly atc Ile	Glu tgc Cys	
<ul><li>35</li><li>40</li><li>45</li></ul>	ggc Gly acc Thr	gag Glu acc Thr 50	Asp ggc Gly 35 ggc Gly	Gly 20 gat Asp aag Lys	gcc Ala ctg Leu	val acc Thr	tac Tyr gtg Val 55	ggc Gly 40 ccc Pro	His 25 aag Lys tgg Trp	ctg Leu ccc Pro	Phe acc Thr acc Thr	ser ctg Leu ctc Leu 60	Val aag Lys 45 gtg Val cac	Ser 30 ttc Phe acc Thr	Gly atc Ile acc Thr	Glu tgc Cys ttc Phe	144
40	ggc Gly acc Thr ggc Gly 65	gag Glu acc Thr 50 tac Tyr	Asp ggc Gly 35 ggc Gly ggc Gly	Gly 20 gat Asp aag Lys ctg Leu	gcc Ala ctg Leu atg Met	acc Thr ccc Pro	Asn tac Tyr gtg Val 55 ttc Phe gcc	Gly ggc Gly 40 ccc Pro gcc Ala	His 25 aag Lys tgg Trp cgc Arg	ctg Leu ccc Pro tac Tyr	Phe acc Thr acc Thr ccc Pro 75	Ser  ctg Leu  ctc Leu 60 gac Asp	Val aag Lys 45 gtg Val cac His	Ser 30 ttc Phe acc Thr atg Met	Gly atc Ile acc Thr aag Lys	Glu tgc Cys ttc Phe cgg Arg 80 cgc	144

Venus F1B F46L, F64L mutations SEQ ID NOS:226 & 227

5							gag Glu										48
10							gta Val										96
15							acc Thr										144
							ccc Pro 55										192
20							tgc Cys										240
25	_		_			_	tcc Ser	_	_		_			_	_		288
30 -				ttc Phe 100		_	_				•						309
35				F46L, 228 &			ıtati	ions	and	pos.	.1 Me	et re	emove	ed			
35 40	SEQ gtg	ID Nagc	10S:2 aag	228 8 ggc	229 gag	gag	ıtati ctg Leu	ttc	acc	ggg	gtg	gtg	ccc	atc			48
40	SEQ gtg Val 1 gag	agc Ser	aag Lys gac	ggc Gly ggc	gag Glu 5 gac	gag Glu gta	ctg	ttc Phe ggc	acc Thr	ggg Gly 10	gtg Val	gtg Val agc	ccc Pro	atc Ile tcc	Leu 15 ggc	Val gag	<b>48</b> 96
	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20 gat	gag Glu 5 gac Asp	gag Glu gta Val	ctg Leu aac	ttc Phe ggc Gly	acc Thr cac His 25	999 Gly 10 aag Lys	gtg Val ttc Phe	gtg Val agc Ser	ccc Pro gtg Val	atc Ile tcc Ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
40	SEQ gtg Val 1 gag Glu ggc Gly acc	agc Ser ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val acc Thr	ctg Leu aac Asn	ttc Phe ggc Gly ggc Gly 40 ccc	acc Thr cac His 25 aag Lys	ggg Gly 10 aag Lys ctg Leu	gtg Val ttc Phe acc Thr	gtg Val agc Ser ctg Leu	ccc Pro gtg Val aag Lys 45	atc Ile tcc Ser 30 ctg Leu	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96

					tcc Ser									288
5		ttc Phe	•	_	_									306
10		F40												
15					gag Glu									48
20					gac Asp									96
25					gcc Ala								atc Ile	1.44
20					ctg Leu									192
30					cag Gln 70									240
35	cgg Arg				aag Lys									288
40	_				aag Lys	_								309
45		F46 NOS:2			ion a	and p	osit	. 1	Met	remo	oved			
					gag Glu									48
50					gta Val									96
55					acc Thr								tgc Cys	144

						gtg Val 55										19	92
5			 _	_	_	ttc Phe	_	_			_		_	_		24	40
10						gcc Ala										28	88
15		atc Ile		_												3(	06
20		F1B ID N													-		
25						gag Glu										4	48
						gta Val										· <u> </u>	96
30 .						acc Thr										14	44
35						ccc Pro 55										19	92
40	_			_	_	tgc Cys		_	_			_		_	_	24	40
45						tcc Ser										28	88
40					aag Lys											30	09
50		F1B ID N				and p	os.	1 Me	et re	emove	ed						
55						ctg Leu										4	48

					gta Val									96
5					acc Thr									144
10					ccc Pro									192
15					tgc Cys 70									240
20					tcc Ser									288
				aag Lys	_									306
25		F1B ID N		mutai k 239										
30					gag Glu									48
35					gac Asp									96
40			 	_	gcc Ala			_	-		_	-		144
10					ctg Leu									192
45					cag Gln 70									240
50					aag		atg Met							288
	9		rne	85	D <sub>1</sub> U			90		•	_		95	

SEQ ID NOS:240 & 241 gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg gtc 48 5 Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag 96 Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu 10 ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc 144 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys 40 45 15 ace ace gge aag etg eee gtg eee tgg eee ace ete gtg ace ace tte 192 Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe 50 55 20 ggc tgg ggc ctg cag tgc ttc gcc cgc tac ccc gac cac atg aag cgg 240 Gly Trp Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Arg 70 cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc 288 25 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arq 85 acc atc ttc ttc aag gac 306 Thr Ile Phe Phe Lys Asp 30 100 35 CFP F1B S65A mutation SEQ ID NOS:242 & 243 atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg 48 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu 40 gtc gag ctg gac qqc qac qta aac qqc cac aaq ttc aqc qtq tcc qqc 96 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly 20 45 gag ggc gag ggc gat gcc acc tac ggc aag ctg acc ctq aaq ttc atc 144

Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile

tgc acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc

Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr

ttc gcc tac ggc ctg cag tgc ttc gcc cgc tac ccc gac cac atg aag

Phe Ala Tyr Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys

55

70

35

50

50

55

CFP F1B Y66W mutation and pos.1 Met removed

75

192

240

					aag Lys										288
5	_				aag Lys	_									309
10	-	F1B ID I	5 <b>A</b> 1		ion	and	pos	. 1 1	Met 1	remov	red .				
15					gag Glu										48
20					gta Val										96
25					acc Thr										144
23					ccc Pro										192
30	-		 _	_	tgc Cys 70		_	_			_	_	_	 ;	240
35					tcc Ser									:	288
40			ttc Phe 100									•		:	306
45			5A, 3 246 8		ano	B S72	2 <b>A</b> mu	ıtati	ions						
					gag Glu										48
50					gac Asp										96
55					gcc Ala										144

	_			aag Lys			_									192
5				ctg Leu												240
10				ttc Phe 85												288
15	_			ttc Phe												309
														*		
20				₹66₩, & 249		i S72	2A mi	ıtati	ions	, and	l pos	sit.	1 Me	et re	emoved	
25				gag Glu 5												48
00				gac Asp												96
30		_	 _	gcc Ala				_	_		_	_			-	144
35			 _	ctg Leu												192
40				cag Gln	_		_	_			_		_	_		240
45				aag Lys 85												288
50				aag Lys												306
50				365T, ≩ 251		1 Y66	5₩ mi	ıtati	ions							
55				ggc Gly 5												48

				gac Asp 20													96
5				ggc Gly													144
10				ggc Gly													192
15				ggc Gly	_	_	_		_	_			_		_	_	240
20				ttc Phe													288
				ttc Phe 100													309
25				4L ,8 252 8			1 Y66	5₩ mι	ıtati	ions,	and	d pos	3. 1	Met	remo	oved	
30				ggc Gly													48
35	gag Glu		qac	ggc		gta		ggc	cac	aaq	ttc	age	~+~	+ 00	aac	gag	96
		пеп			Asp	Vai	Asn	Gly									
40		gag	Asp ggc	Gly	gcc	acc	tac	ggc	His 25 aag	Lys ctg	Phe-	Ser	Val aag	Ser 30 ttc	Gly	Glu tgc	144
40	Gly	gag Glu acc	Asp ggc Gly 35 ggc	Gly 20 gat	gcc Ala ctg	acc Thr	tac Tyr gtg	ggc Gly 40	His 25 aag Lys tgg	Lys ctg Leu ccc	Phe- acc Thr	ser ctg Leu	Val aag Lys 45	Ser 30 ttc Phe	Gly atc Ile	Glu tgc Cys	144
40 45	Gly acc Thr	gag Glu acc Thr 50	Asp ggc Gly 35 ggc Gly	Gly 20 gat Asp	gcc Ala ctg Leu	acc Thr ccc Pro	tac Tyr gtg Val 55	ggc Gly 40 ccc Pro	His 25 aag Lys tgg Trp	ctg Leu ccc Pro	Phe- acc Thr acc Thr	ctc Leu ctc Leu 60	aag Lys 45 gtg Val	Ser 30 ttc Phe acc Thr	atc Ile acc Thr	Glu tgc Cys ctc Leu	
	acc Thr acc Thr 65	gag Glu acc Thr 50 tgg Trp	Asp ggc Gly 35 ggc Gly ggc Gly	Gly 20 gat Asp aag Lys	gcc Ala ctg Leu cag Gln	acc Thr ccc Pro tgc Cys 70	tac Tyr gtg Val 55 ttc Phe	ggc Gly 40 ccc Pro gcc Ala	His 25 aag Lys tgg Trp cgc Arg	ctg Leu ccc Pro tac Tyr	Phe- acc Thr acc Thr ccc Pro 75	Ser  ctg Leu  ctc Leu 60 gac Asp	Val aag Lys 45 gtg Val cac His	Ser 30 ttc Phe acc Thr atg Met	atc Ile acc Thr aag Lys	Glu tgc Cys ctc Leu cgg Arg 80 cgc	192

## BFP F1B, Y66H mutation SEQ ID NOS:254 & 255

5							ctg Leu									48
10							aac Asn									96
15							tac Tyr 40									144
.0							gtg Val									192
20							ttc Phe									240
25							gcc Ala									288
30	_	acc Thr			_	_								·		309
35		F1B ID I				and	pos	.1 M∈	et re	emove	ed					
40							ttc Phe									48
45							ggc Gly									96
40							ggc Gly 40									144
50			~~~	 	000	~+~	ccc	taa	CCC	acc	ctc	ata	acc	acc	ttc	192
							Pro									232

				ttc Phe													288
5				ttc Phe 100	_	_											306
10				64L, 258 a			tatio	ons									
15				aag Lys													48
20				gac Asp 20													96
				ggc Gly													144
25				ggc Gly													192
30	ctg Leu 65	ggc Gly	cac His	ggc Gly	ctg Leu	cag Gln 70	tgc Cys	ttc Phe	gcc Ala	cgc Arg	tac Tyr 75	ccc Pro	gac Asp	cac His	atg Met	aag Lys 80	240
35				ttc Phe													288
40				ttc Phe 100													309
45				54L, 260 8			tatio	ns a	and p	os.1	L Met	ren	nove	i			٠
				ggc Gly													48
50				ggc Gly 20													96
55				gat Asp													144

					gtg Val 55										. 1	92
5					ttc Phe											40
10					gcc Ala										2	88
15	atc Ile		_	_										٠	3	06
20	F2B ID N				1-24(	of	EYFI	?) M∈	et ad	ded	@ I	pos.	1 .			
					acc Thr									gac Asp		48
25					gag Glu											96
30					aag Lys										1	44
35					aag Lys 55										1	92
40					gag Glu										2	40
					atc Ile										2	88
45					cag Gln										3:	36
50			_	_	ctg Leu						_	_			3	84
55					ctg Leu 135										4	11

EGFP F2B(emerald), N146K, M153T, and I167T mutations SEQ ID NOS:264 & 265

5													gac Asp 15			48
10													gac Asp			96
15		_		_	_	_					_		aag Lys	_		144
										_			ttc Phe	_		192
20													cac His			240
25													gac Asp 95			288
30													gag Glu			336
35													atc Ile			384
40				ctg Leu		_										408
40																
45		3(eme NOS:2			(, M)	.53T,	I167	7 <b>T</b> mu	ıtati	.ons	and	Met	adde	ed @ j	pos.	1
													ggc Gly 15			48
50													gag Glu			96-
55													cac His			144

						gac Asp											19	2
5						atc Ile 70											24	0
10						ccc Pro											28	8
15				_	_	acc Thr	_		_	_	_		_				33	6
20						gtc Val											38	4
20				_	_	gag Glu	_		_		•						41	1
25																		
		F2B		03F r	nutat	ion												
20	SEQ	ID 1	NOS : 2	268 8	ž 269	9												
30	gac	ggc	aac	tac	aag	acc Thr			_						_		4	8
35	gac Asp 1	ggc Gly gtg	aac Asn	tac Tyr cgc	aag Lys 5	acc	Arg ctg	Ala	Glu	Val 10 atc	Lys gac	Phe ttc	Glu	Gly	Asp 15 gac	Thr ggc	. 9	
	gac Asp 1 ctg Leu	ggc Gly gtg Val	aac Asn aac Asn	tac Tyr cgc Arg 20	aag Lys 5 atc Ile	acc Thr	Arg ctg Leu ctg	Ala aag Lys gag	Glu ggc Gly 25 tac	Val 10 atc Ile aac	Lys gac Asp	Phe ttc Phe	Glu aag Lys agc	Gly gag Glu 30 cac	Asp 15 gac Asp	Thr ggc Gly gtc		6
35	gac Asp 1 ctg Leu aac Asn	ggc Gly gtg Val atc Ile	aac Asn aac Asn ctg Leu 35	tac Tyr cgc Arg 20 ggg Gly	aag Lys 5 atc Ile cac His	acc Thr gag Glu	ctg Leu ctg Leu	aag Lys gag Glu 40	Glu ggc Gly 25 tac Tyr	Val 10 atc Ile aac Asn	Lys gac Asp tac Tyr	Phe ttc Phe aac Asn	Glu aag Lys agc Ser 45	Gly gag Glu 30 cac His	Asp 15 gac Asp aac Asn	Thr  ggc Gly  gtc Val  aag	9	6
35 40 45	gac Asp 1 ctg Leu aac Asn tat Tyr	ggc Gly gtg Val atc Ile atc Ile 50	aac Asn aac Asn ctg Leu 35 atg Met	tac Tyr cgc Arg 20 ggg Gly gcc Ala	aag Lys 5 atc Ile cac His gac Asp	acc Thr gag Glu aag Lys	ctg Leu ctg Leu cag Gln 55	Ala aag Lys gag Glu 40 aag Lys	ggc Gly 25 tac Tyr aac Asn	Val 10 atc Ile aac Asn ggc Gly	gac Asp tac Tyr atc Ile	Phe ttc Phe aac Asn aag Lys 60 ctc	Glu aag Lys agc Ser 45 gtg Val	Gly gag Glu 30 cac His aac Asn	Asp 15 gac Asp aac Asn ttc Phe	Thr  ggc Gly  gtc Val  aag Lys tac	9	6
35 40	gac Asp 1 ctg Leu aac Asn tat Tyr atc Ile 65 cag	ggc Gly gtg Val atc Ile atc Ile 50 cgc Arg	aac Asn aac Asn ctg Leu 35 atg Met	tac Tyr cgc Arg 20 ggg Gly gcc Ala aac Asn	aag Lys 5 atc Ile cac His gac Asp	acc Thr gag Glu aag Lys aag Lys	ctg Leu ctg Leu cag Gln 55 gac Asp	Ala aag Lys gag Glu 40 aag Lys ggc Gly gac	ggc Gly 25 tac Tyr aac Asn agc ser	Val 10 atc Ile aac Asn ggc Gly gtg Val	gac Asp tac Tyr atc Ile cag Gln 75	Phe ttc Phe aac Asn aag Lys 60 ctc Leu ctg	aag Lys agc Ser 45 gtg Val gcc Ala	gag Glu 30 cac His aac Asn	Asp 15 gac Asp aac Asn ttc Phe cac His	Thr  ggc Gly  gtc Val  aag Lys  tac Tyr 80  aac	14 19	6 4 2

5				gtc Val											384
•			_	 gag Glu	-		_								408
10		F2B ID 1		nutat § 27:		and	Met	adde	ed @	pos.	. 1				
15				tac Tyr 5											48
20		_		cgc Arg			_	_			_		-	 _	96
25				ggg ggg											144
23				gcc Ala											192
30				aac Asn											240
35	tac Tyr			acc Thr 85											288
40				agc Ser					-	_		_			336
45				atg Met											384
				gac Asp											411
50				nutat k 273				٠							
55				aag Lys 5											48

	gtg Val													96	5
5	atc Ile	_		_	_						_		gtc Val	144	1
10	atc Ile 50													192	2
15	cgc Arg													240	)
20	cag Gln													288	3
	tac Tyr												aag Lys	336	5
25	gat Asp													384	1
30	ggc Gly 130													408	3
35	F2B ID N				and	Met	adde	ed @	pos.	. 1					•
40	gac Asp													48	3
45	ctg Leu													96	5
50	aac Asn													144	1
	tat Tyr 50													192	2
55	atc Ile													240	)

5		aac Asn								288
		ctg Leu 100								336
10		cac His								384
15		atg Met								411
20	 F2B ID 1	16I r 276 8			٠					
25		tac Tyr								48
20		cgc Arg 20								96
30		ggg Gly								144
35		gcc Ala								192
40		aac Asn								240
45		acc Thr								288
		agc Ser 100	_	_	_	_	_		 -	336
50		atg Met								384
55		gac Asp								408

SEQ ID NOS:278 & 279 atg gac ggc aac tac aag acc cgc gcc gag gtg aag ttc gag ggc gac 48 Met Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag gag gac 96 10 Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp qqc aac atc ctq qqq cac aaq ctq qaq tac aac tac atc aqc cac aac 144 Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Ile Ser His Asn 15 35 gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg aac ttc 192 Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn Phe 55 20 aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc gac cac 240 Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp His 70 25 tac cag cag aac acc ccc atc ggc gac ggc ccc gtg ctg ctc gac 288 Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp 90 aac cac tac ctg agc acc cag tcc gcc ctg agc aaa gac ccc aac gag 336 30 Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu 100 105 aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc gcc gcc ggg atc 384 Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile 35 115 120 act ctc ggc atg gac gag ctg tac aag 411 Thr Leu Gly Met Asp Glu Leu Tyr Lys 130 40 CFP F2B M153T mutation 45 SEQ ID NOS:280 & 281 gac ggc aac tac aag acc cgc gcc gag gtg aag ttc gag ggc gac acc 48 Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr 10 50 ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag gag gac ggc 96 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly 20 55 aac atc ctg ggg cac aag ctg gag tac aac tac aac agc cac aac gtc 144 Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val

CFP F2B N146I mutation and Met added @ pos. 1

40

35

5						aag Lys											192
						gag Glu 70											240
10						atc Ile											288
15						cag Gln											336
20				Met		ctg Leu											384
25						ctg Leu											408
		F2B ID N				cion	and	Met	ado	ded (	o pos	3. 1					
						-											
30		gac	ggc	aac	tac	aag Lys											48
	Met 1 acc	gac Asp ctg	ggc Gly gtg	aac Asn aac	tac Tyr 5	aag Lys	Thr gag	Arg ctg	Ala aag	Glu 10 ggc	Val atc	Lys gac	Phe ttc	Glu aag	Gly 15 gag	Asp gac	48 96
	Met 1 acc Thr	gac Asp ctg Leu	ggc Gly gtg Val	aac Asn aac Asn 20	tac Tyr 5 cgc Arg	aag Lys atc	Thr gag Glu aag	Arg ctg Leu ctg	Ala aag Lys 25 gag	Glu 10 ggc Gly tac	val atc Ile	Lys gac Asp	Phe ttc Phe aac	Glu aag Lys 30	Gly 15 gag Glu cac	gac Asp	
35 40	Met 1 acc Thr ggc Gly	gac Asp ctg Leu aac Asn	ggc Gly gtg Val atc Ile 35	aac Asn aac Asn 20 ctg Leu	tac Tyr 5 cgc Arg ggg Gly	aag Lys atc Ile	Thr gag Glu aag Lys	ctg Leu ctg Leu 40	Ala aag Lys 25 gag Glu aag	Glu 10 ggc Gly tac Tyr	val atc Ile aac Asn	Lys gac Asp tac Tyr	Phe ttc Phe aac Asn 45	aag Lys 30 agc Ser	Gly 15 gag Glu cac His	Asp gac Asp aac Asn	96
35	Met 1 acc Thr ggc Gly gtc Val aag	gac Asp ctg Leu aac Asn tat Tyr 50	ggc Gly gtg Val atc Ile 35 atc Ile	aac Asn 20 ctg Leu act Thr	tac Tyr 5 cgc Arg ggg Gly gcc Ala	aag Lys atc Ile cac His	Thr gag Glu aag Lys aag Lys 55	ctg Leu ctg Leu 40 cag Gln	Ala aag Lys 25 gag Glu aag Lys	Glu 10 ggc Gly tac Tyr aac Asn	val atc Ile aac Asn ggc Gly	gac Asp tac Tyr atc 11e 60 cag	Phe ttc Phe aac Asn 45 aag Lys	Glu aag Lys 30 agc ser gtg Val	Gly 15 gag Glu cac His aac Asn	gac Asp aac Asn ttc Phe	96 144
35 40	Met 1 acc Thr ggc Gly gtc Val aag Lys 65 tac	gac Asp ctg Leu aac Asn tat Tyr 50 atc Ile	ggc Gly gtg Val atc Ile 35 atc Ile cgc Arg	aac Asn 20 ctg Leu act Thr	tac Tyr 5 cgc Arg ggg Gly gcc Ala aac Asn	aag Lys atc Ile cac His gac Asp	Thr gag Glu aag Lys 55 gag Glu atc	ctg Leu ctg Leu 40 cag Gln gac Asp	Ala aag Lys 25 gag Glu aag Lys ggc Gly	Glu 10 ggc Gly tac Tyr aac Asn agc ser	val atc Ile aac Asn ggc Gly gtg val 75 ccc	Lys gac Asp tac Tyr atc ile 60 cag Gln gtg	Phe ttc Phe aac Asn 45 aag Lys ctc Leu ctg	aag Lys 30 agc ser gtg Val gcc Ala	Gly 15 gag Glu cac His aac Asn gac Asp	gac Asp aac Asn ttc Phe cac His 80 gac	96 144 192

											gtg Val						384
5		ctc Leu 130															411
10		F2B ID I					nutat	ions	5								
15											aag Lys						48
20											gac Asp						96
											tac Tyr						144
25											atc Ile						192
30											cag Gln 75						240
35											gtg Val						288
40											aaa Lys						336
.0	cgc Arg	gat Asp	cac His 115	atg Met	gtc Val	ctg Leu	Leu	gag Glu 120	ttc Phe	gtg Val	acc Thr	gcc Ala	gcc Ala 125	gly ggg	atc Ile	act Thr	384
45		ggc Gly 130															408
50		F2B ID N					atio	ons a	and N	1et a	added	d @ r	os.	1			
55											gtg Val						48

					atc Ile									96
5					cac His									144
10					gac Asp									192
15					atc Ile 70									240
20					ccc Pro									288
			_	_	acc Thr	_		_	_	_	_			336
25					gtc Val									384
30			_	_	gag Glu	_		_						411
35	F2B ID 1				ation 9	1								
40					acc Thr									48
45					gag Glu									96
	Ile	_			aag Lys	_	-				_		-	144
50					aag Lys									192
55					gag Glu 70									240

														ccc Pro		288
5														aac Asn 110		336
10														ggg Gly		384
15						Leu	tac Tyr 135	_							·	408
20			V16 NOS:2				n and	i Met	ado	ded (	pos	sit.	1			
														gag Glu		48
25														aag Lys 30		96
30														agc Ser		144
35														gcc Ala		192
40														gcc Ala		240
.0														ctg Leu		288
45				_	_		_		_	_	_		_	ccc Pro 110		 336
50	_	_	_		_	_	_	-						gcc Ala		384
55							ctg Leu 135									411

CFP F2B N146I,V163A mutations SEQ ID NOS:292 & 293

5				acc Thr												48
10				gag Glu	_	-			_		_		_			96
15		_		aag Lys	_						_			_		144
				aag Lys												192
20				gag Glu 70												240
25				atc Ile												288
30				cag Gln												336
35				ctg Leu												384
				ctg Leu												408
40					·											
45			/163 <i>I</i> 295	A mut	atio	ons a	and N	Met a	added	d @ p	os.	1				
				aag Lys												48
50				atc Ile												96
55				cac His											•	144

		tat Tyr 50																192
5	_	atc Ile	_					_		_		_		_	_			240
10		cag Gln	_						_				_	_		-	;	288
15		cac His		_	_		_		_	_	_						•	336
20		cgc Arg							Glu								:	384
		ctc Leu 130											. •					411
25																		
	CFP	F2B	M15	53T,V	/163/	A mut	atio	ons										
30 .	SEQ	ID 1	10S:2	296 8	£ 29′	7												
30	gac	ggc Gly	aac	tac	aag	acc												48
35	gac Asp 1	ggc	aac Asn	tac Tyr cgc	aag Lys 5 atc	acc Thr	Arg ctg	Ala aag	Glu ggc	Val 10 atc	Lys gac	Phe ttc	Glu aag	Gly gag	Asp 15 gac	Thr ggc		48
	gac Asp 1 ctg Leu	ggc Gly gtg	aac Asn aac Asn	tac Tyr cgc Arg 20	aag Lys 5 atc Ile	acc Thr gag Glu	Arg ctg Leu ctg	Ala aag Lys gag	Glu ggc Gly 25 tac	Val 10 atc Ile aac	Lys gac Asp	Phe ttc Phe aac	Glu aag Lys agc	Gly gag Glu 30 cac	Asp 15 gac Asp	Thr ggc Gly gtc		
35	gac Asp 1 ctg Leu aac Asn	ggc Gly gtg Val	aac Asn aac Asn ctg Leu 35	tac Tyr cgc Arg 20 ggg Gly	aag Lys 5 atc Ile cac His	acc Thr gag Glu aag Lys	ctg Leu ctg Leu	aag Lys gag Glu 40	ggc Gly 25 tac Tyr	Val 10 atc Ile aac Asn	gac Asp tac Tyr	Phe ttc Phe aac Asn	aag Lys agc Ser 45	gag Glu 30 cac His	Asp 15 gac Asp aac Asn	Thr  ggc Gly  gtc Val  aag		96
35 40 45	gac Asp 1 ctg Leu aac Asn tat Tyr	ggc Gly gtg Val atc Ile	aac Asn ctg Leu 35 acc Thr	tac Tyr cgc Arg 20 ggg Gly gcc Ala	aag Lys 5 atc Ile cac His gac Asp	acc Thr gag Glu aag Lys aag Lys	ctg Leu ctg Leu cag Gln 55	Ala aag Lys gag Glu 40 aag Lys	ggc Gly 25 tac Tyr aac Asn	Val 10 atc Ile aac Asn ggc Gly	gac Asp tac Tyr atc Ile	Phe ttc Phe aac Asn aag Lys 60 ctc	Glu aag Lys agc Ser 45 gcc Ala	gag Glu 30 cac His aac Asn	Asp 15 gac Asp aac Asn ttc Phe	Thr  ggc Gly  gtc Val  aag Lys tac		96 144
35	gac Asp 1 ctg Leu aac Asn tat Tyr atc Ile 65 cag	ggc Gly gtg Val atc Ile atc Ile 50	aac Asn aac Asn ctg Leu 35 acc Thr	tac Tyr cgc Arg 20 ggg Gly gcc Ala aac Asn	aag Lys 5 atc Ile cac His gac Asp	acc Thr gag Glu aag Lys aag Lys gag Glu 70 atc	ctg Leu ctg Leu cag Gln 55 gac Asp	Ala aag Lys gag Glu 40 aag Lys ggc Gly	ggc Gly 25 tac Tyr aac Asn agc ser	Val 10 atc Ile aac Asn ggc Gly gtg Val	gac Asp tac Tyr atc Ile cag Gln 75 gtg	Phe ttc Phe aac Asn aag Lys 60 ctc Leu ctg	Glu aag Lys agc Ser 45 gcc Ala gcc Ala	gag Glu 30 cac His aac Asn	Asp 15 gac Asp aac Asn ttc Phe cac His	Thr  ggc Gly  gtc Val  aag Lys  tac Tyr 80  aac		96 144 192

5										gtg Val							384
					_	_	tac Tyr 135	_									408
10					163A & 299		ation	ns ar	nd Me	et ad	ded	@ po	os. :	1			
15										gag Glu 10							48
20										ggc Gly							96
25	ggc Gly	aac Asn	atc Ile 35	ctg Leu	999 Gly	cac His	aag Lys	ctg Leu 40	gag Glu	tac Tyr	aac Asn	tac Tyr	aac Asn 45	agc Ser	cac His	aac Asn	144
										aac Asn							192
30										agc Ser							240
35										ggc Gly 90							288
40										ctg Leu							336
45										ttc Phe							3,84
			Gly				ctg Leu 135	Tyr									411
50					M153		nd V1	.63A	muta	atior	ıs						
55										gtg Val 10							48

				gag Glu											96
5				aag Lys											144
10				aag Lys											192
15				gag Glu 70											240
20				atc Ile											288
		_	_	cag Gln		_	_	_		_				_	336
25				ctg Leu											384
30		_	_	 ctg Leu		_									408
35		, NI NOS:3			nd V	163A	muta	ation	ns ar	nd Me	et ad	lded	@ po	os. 1	
40				aag Lys											48
45				atc Ile											96
50				cac His											144
				gac Asp											192
55				atc Ile 70											240

5			ccc Pro							:	288
			acc Thr							:	336
10			gtc Val								384
15			gag Glu							•	411
20		N146: 304 8	203Y 5	muta	ation	ns					
25			acc Thr								48
			gag Glu								96
30			aag Lys							:	144
35			aag Lys							:	192
40			gag Glu 70							:	240
45			atc Ile							:	288
			cag Gln								336
50			ctg Leu							:	384
55			ctg Leu							. 4	408

		306 8		7	nucai	. 1.011	s and	J Me	. au	ieu (	s po:	o. 1			
5				aag Lys											48
10				atc Ile											96
15				cac His											144
20				gac Asp											192
				atc Ile 70										cac His 80	240
25				ccc Pro											288
30				tac Tyr											336
35				gtc Val											384
40				gag Glu											411
45		L53T, 308 &		)3Y n	nutat	ions	3								
			_	acc Thr	_	_			_				_		48
50				gag Glu											96
55				aag Lys											144

CGFP F2B , N146I,T203Y mutations and Met added @ pos. 1  $\,$ 

						aag Lys											192
5						gag Glu 70											240
10						atc Ile											288
15						cag Gln											336
20						ctg Leu											384
						ctg Leu											408
25																	
			3 , I	11537	Г, Т2	203Y	muta	ation	ns +	Met	@ pc	sit.	,				
30	SEQ	ID 1	10s:	310 8	x 311	1											
30	atg	gac	ggc	aac	tac	l aag Lys											48
35	atg Met 1	gac Asp ctg	ggc Gly gtg	aac Asn aac	tac Tyr 5 cgc	aag	Thr gag	Arg ctg	Ala aag	Glu 10 ggc	Val atc	Lys gac	Phe-	Glu aag	Gly 15 gag	Asp gac	96
	atg Met 1 acc Thr	gac Asp ctg Leu	ggc Gly gtg Val	aac Asn aac Asn 20	tac Tyr 5 cgc Arg	aag Lys atc	Thr gag Glu aag	Arg ctg Leu ctg	Ala aag Lys 25 gag	Glu 10 ggc Gly tac	Val atc Ile aac	Lys gac Asp tac	Phe- ttc Phe	Glu aag Lys 30	Gly 15 gag Glu cac	Asp gac Asp	-
35	atg Met 1 acc Thr ggc Gly	gac Asp ctg Leu aac Asn	ggc Gly gtg Val atc Ile 35	aac Asn aac Asn 20 ctg Leu	tac Tyr 5 cgc Arg ggg Gly	aag Lys atc Ile	Thr gag Glu aag Lys aag	ctg Leu ctg Leu 40	Ala aag Lys 25 gag Glu aag	Glu 10 ggc Gly tac Tyr	Val atc Ile aac Asn	Lys gac Asp tac Tyr	Phettc Phetaac Asn 45	aag Lys 30 agc Ser	Gly 15 gag Glu cac His	gac Asp aac Asn	96
35 40 45	atg Met 1 acc Thr ggc Gly gtc Val	gac Asp ctg Leu aac Asn tat Tyr 50	ggc Gly gtg Val atc Ile 35 atc Ile	aac Asn 20 ctg Leu acc Thr	tac Tyr 5 cgc Arg ggg Gly gcc Ala	aag Lys atc Ile cac His gac Asp	Thr  gag Glu  aag Lys aag Lys 55	ctg Leu ctg Leu 40 cag Gln	Ala aag Lys 25 gag Glu aag Lys	Glu 10 ggc Gly tac Tyr aac Asn	Val atc Ile aac Asn ggc Gly gtg	gac Asp tac Tyr atc Ile 60	Phettc Phe aac Asn 45 aag Lys	Glu aag Lys 30 agc ser gtg Val	Gly 15 gag Glu cac His aac Asn	gac Asp aac Asn ttc Phe	96 144
35 40 45	atg Met 1 acc Thr ggc Gly gtc Val aag Lys 65	gac Asp ctg Leu aac Asn tat Tyr 50 atc Ile	ggc Gly gtg Val atc Ile 35 atc Ile cgc Arg	aac Asn 20 ctg Leu acc Thr	tac Tyr 5 cgc Arg ggg Gly gcc Ala aac Asn	aag Lys atc Ile cac His gac Asp	Thr  gag Glu  aag Lys aag Lys 55 gag Glu atc	ctg Leu ctg Leu 40 cag Gln gac Asp	Ala aag Lys 25 gag Glu aag Lys ggc Gly	Glu 10 ggc Gly tac Tyr aac Asn agc ser	Val atc Ile aac Asn ggc Gly gtg Val 75 ccc	Lys gac Asp tac Tyr atc ile 60 cag Gln gtg	Phettc Phetaac Asn 45 aag Lys ctc Leu ctg	aag Lys 30 agc Ser gtg Val gcc Ala	Gly 15 gag Glu cac His aac Asn	gac Asp aac Asn ttc Phe cac His 80 gac	96 144 192

5								gtg Val							384
Ü				ctg Leu 135											411
10	P F21			and	T20:	3Y mi	ıtat:	ions						•	
15								aag Lys							48
20								gac Asp							96
								tac Tyr							144
25								atc Ile							192
30								cag Gln 75							240
35								gtg Val					aac Asn		288
40								aaa Lys							336
				Leu				acc Thr							384
45				tac Tyr 135	_										408
50	P F2I			nd T2	203Y	muta	ation	ns ar	nd Me	et ac	dded	@ pc	os. 1		
55								gtg Val							48

										ggc Gly				96
5				_			_	_		tac Tyr		_		144
10										aac Asn				192
15										agc Ser				240
20										ggc Gly 90				288
										ctg Leu				336
25										ttc Phe				384
30						gag Glu								411
35				/163 <i>I</i> 316 8		203Y 7	muta	ation	ns					
40	gac	ggc	aac	tac	aag	acc				gtg Val 10				48
45										atc Ile				96
50										aac Asn				144
55										ggc Gly				192
55										gtg Val				240

5			acc Thr													288
			agc Ser 100													336
10			atg Met													384
15		_	gac Asp		_		_									408
20			/163 <i>I</i> 318 8			muta	ation	ıs ar	nd Me	et ad	ided	@ pc	os. I	L .		
25			aac Asn													48
			aac Asn 20													96
30			ctg Leu													144
35			atg Met													192
40			cac His													240
45			aac Asn													288
			ctg Leu 100	_		_		_		_		_				336
50			cac His													384
55			atg Met													411

CGFP F2B, N146I, V163A, and T203Y mutations SEQ ID NOS:320 & 321

5	_				_		cgc Arg	_			_				_		48
10							ctg Leu										96
15							ctg Leu										144
			_	_	_	_	cag Gln 55	_				_	_			_	192
20							gac Asp										240
25							ggc Gly										288
30							tcc Ser										336
35							ctg Leu										384
							tac Tyr 135										408
40																-	
45		P F2E					nd T2	203Y	muta	ation	ns ar	nd Me	et ac	ided	@ pc	os. 1	
							acc Thr										48
50							gag Glu										96
55							aag Lys										144

				atg Met													192
5				cac													240
10				aac Asn													288
15				ctg Leu 100													336
20				cac His													384
				atg Met													411
25																	
	CGFI	P F2I	3. M:	153T	. V16	53A.	and	T203	3Y mi	ıtati	ions						
30				324 8													
30	SEQ gac	ID N	NOS:		325 aag	acc	cgc	gcc	gag	gtg	aag						48
35	gac Asp 1	ID Maggc Gly	aac Asn aac	324 8 tac	aag Lys 5	acc Thr	cgc Arg	gcc Ala aag	gag Glu ggc	gtg Val 10 atc	aag Lys gac	Phe ttc	Glu aag	Gly	Asp 15 gac	Thr ggc	48 96
	gac Asp 1 ctg Leu	ggc Gly gtg Val	aac Asn aac Asn	tac Tyr cgc Arg	aag Lys 5 atc Ile	acc Thr gag Glu	cgc Arg ctg Leu	gcc Ala aag Lys	gag Glu ggc Gly 25 tac	gtg Val 10 atc Ile	aag Lys gac Asp	Phe ttc Phe aac	Glu aag Lys agc	Gly gag Glu 30 cac	Asp 15 gac Asp	Thr ggc Gly gtc	
35	gac Asp 1 ctg Leu aac Asn	ggc Gly gtg Val atc Ile	aac Asn aac Asn ctg Leu 35	tac Tyr cgc Arg 20	aag Lys 5 atc Ile cac His	acc Thr gag Glu aag Lys	cgc Arg ctg Leu ctg Leu	gcc Ala aag Lys gag Glu 40	gag Glu ggc Gly 25 tac Tyr	gtg Val 10 atc Ile aac Asn	aag Lys gac Asp tac Tyr	Phe ttc Phe aac Asn	aag Lys agc Ser 45	gag Glu 30 cac His	Asp 15 gac Asp aac Asn	Thr  ggc Gly  gtc Val  aag	96
35 40 45	gac Asp 1 ctg Leu aac Asn tat Tyr	ggc Gly gtg Val atc Ile atc Ile 50 cgc	aac Asn aac Asn ctg Leu 35 acc Thr	tac Tyr cgc Arg 20 999 Gly	aag Lys 5 atc Ile cac His gac Asp	acc Thr gag Glu aag Lys aag Lys	cgc Arg ctg Leu ctg Leu cag Gln 55	gcc Ala aag Lys gag Glu 40 aag Lys	gag Glu ggc Gly 25 tac Tyr aac Asn	gtg Val 10 atc Ile aac Asn ggc Gly	aag Lys gac Asp tac Tyr atc Ile	Phe ttc Phe aac Asn aag Lys 60 ctc	Glu aag Lys agc Ser 45 gcc Ala	Gly gag Glu 30 cac His aac Asn	Asp 15 gac Asp aac Asn ttc Phe	Thr  ggc Gly  gtc Val  aag Lys	96
35 40	gac Asp 1 ctg Leu aac Asn tat Tyr atc Ile 65 cag	ggc Gly gtg Val atc Ile atc Ile 50 cgc Arg	aac Asn aac Asn ctg Leu 35 acc Thr cac His	tac Tyr cgc Arg 20 ggg Gly gcc Ala	aag Lys 5 atc Ile cac His gac Asp atc	acc Thr gag Glu aag Lys aag Lys gag Glu 70 atc	cgc Arg ctg Leu ctg Leu cag Gln 55 gac Asp	gcc Ala aag Lys gag Glu 40 aag Lys ggc Gly	gag Glu ggc Gly 25 tac Tyr aac Asn	gtg Val 10 atc Ile aac Asn ggc Gly gtg Val	aag Lys gac Asp tac Tyr atc Ile cag Gln 75	Phe ttc Phe aac Asn aag Lys 60 ctc Leu ctg	Glu aag Lys agc Ser 45 gcc Ala gcc Ala	gag Glu 30 cac His aac Asn	Asp 15 gac Asp aac Asn ttc Phe cac His	Thr  ggc Gly  gtc Val  aag Lys  tac Tyr 80  aac	96 144 192

5			gtc Val	ctg Leu										384
				tac Tyr 135		-							٠	408
10			,V16:	nd Ti	203Y	muta	atio	ns ai	nd Me	et ad	dded	@ po	os. 1	
15				acc Thr										48
20				gag Glu										96
25				aag Lys										144
				aag Lys 55										192
30 .				gag Glu										240
35				atc Ile										288
40				cag Gln										336
45				ctg Leu										384
				ctg Leu 135										411
50			M153	L63A,	and	T203	3Υ mι	ıtati	.ons	•				
55				cgc Arg										48

										atc Ile								96
5										aac Asn								144
10										ggc Gly								192
15										gtg Val								240
20										ccc Pro 90								288
										agc Ser						aag Lys		336
25										gtg Val								384
	ata	~~~																408
30						ctg Leu												400
35	Leu	Gly 130 P F2F	Met	Asp 1461,	Glu M153	Leu BT,V1	Tyr 135	Lys	T203	3Y mu	ıtati	lons	and	Met	adde	ed @	pos.	
	Leu CGFI SEQ atg	Gly 130 PF2E ID N	Met  Nos:	Asp 1461, 330 & aac	M153 331 tac	Leu BT,V1	Tyr 135 163A,	Lys and	gcc	3Y mu gag Glu 10	gtġ	aag	ttc	gag	ggc	gac.	pos.	
35	CGFF SEQ atg Met 1	Gly 130 P F2F ID N gac Asp	Met  3, Ni NOS:  ggc Gly gtg	Asp L46I, 330 & aac Asn	M153 333 tac Tyr 5	Leu BT,VI aag Lys	Tyr 135 L63A, acc Thr	and cgc Arg	gcc Ala aag	gag Glu	gtġ Val atc	aag Lys gac	ttc Phe ttc	gag Glu aag	ggc Gly 15	gac Asp gac	pos.	1
35 40 45	CGFF SEQ atg Met 1 acc Thr	Gly 130 P F2E ID N gac Asp ctg Leu	Met  3, N: NOS: ggc Gly gtg Val atc	Asp 1461, 330 & aac Asn aac Asn 20	M153  x 333  tac Tyr 5  cgc Arg	Leu BT,VI aag Lys atc Ile	Tyr 135 63A, acc Thr gag Glu	and cgc Arg ctg Leu ctg	gcc Ala aag Lys 25	gag Glu 10 ggc	gtg Val atc Ile	aag Lys gac Asp	ttc Phe ttc Phe	gag Glu aag Lys 30	ggc Gly 15 gag Glu	gac Asp gac Asp	pos.	1 48
35 40	CGFI SEQ atg Met 1 acc Thr	Gly 130 PF2H ID N gac Asp ctg Leu aac Asn	Met  3, N: NOS:  990 Gly  gtg Val  atc Ile 35 atc	Asp 1461, 330 8 aac Asn aac Asn 20 ctg Leu	M153 k 333 tac Tyr 5 cgc Arg ggg Gly	Leu 3T,VI aag Lys atc Ile cac His	Tyr 135 63A, acc Thr gag Glu aag Lys	and cgc Arg ctg Leu ctg Leu 40 cag	gcc Ala aag Lys 25 gag Glu aag	gag Glu 10 ggc Gly tac Tyr	gtġ Val atc Ile aac Asn	aag Lys gac Asp tac Tyr	ttc Phe ttc Phe atc Ile 45	gag Glu aag Lys 30 agc Ser	ggc Gly 15 gag Glu cac His	gac Asp gac Asp aac Asn		1 48

5								ctg Leu		2	88
-								ccc Pro 110		. 3	36
10								gcc Ala		3	84
15				 ctg Leu 135	_					4	11
20			mutai § 33:						•		
25								ggc Gly			48
								gag Glu 30			96
30								cac His		1	44
35								aac Asn		1	92
40								gac Asp		2	40
45								ccc Pro		2	88
								aac Asn 110		3	36
50								ggg Gly		3	84
55				tac Tyr 135						4	80

		F2B,					and	Met	adde	ed @	pos	. 1				
5		gac Asp														48
10		ctg Leu														96
15		aac Asn														144
20		tat Tyr 50														192
		atc Ile													cac His 80	240
25		cag Gln														288
30		cac His		_	_		_		_	_	_		_			336
35	aag Lys	cgc Arg														384
40		ctc Leu 130		_	_		_		_							411
45		ıs F2 ID N	-				on									
		ggc Gly														48
50		gtg Val														96
55		atc Ile				_	_								_	144

5							cag Gln 55										192
							gac Asp										240
10							ggc Gly										288
15							tcc Ser										336
20							ctg Leu					Ala					384
25							tac Tyr 135										408
					muta k 339		n and	d Met	ado	ded @	pos	3. 1					
	-																
30	atg	gac	ggc	aac	tac	aag	acc Thr										48
30	atg Met 1	gac Asp ctg	ggc Gly gtg	aac Asn aac	tac Tyr 5	aag Lys atc		Arg ctg	Ala aag	Glu 10 ggc	Val atc	Lys gac	Phe ttc	Glu aag	Gly 15 gag	Asp gac	48 96
	atg Met 1 acc Thr	gac Asp ctg Leu	ggc Gly gtg Val	aac Asn aac Asn 20	tac Tyr 5 cgc Arg	aag Lys atc Ile	Thr gag	Arg ctg Leu ctg	Ala aag Lys 25 gag	Glu 10 ggc Gly tac	Val atc Ile aac	Lys gac Asp	Phe ttc Phe aac	Glu aag Lys 30 agc	Gly 15 gag Glu cac	gac Asp	
35 40	atg Met 1 acc Thr ggc Gly	gac Asp ctg Leu aac Asn	ggc Gly gtg Val atc Ile 35	aac Asn aac Asn 20 ctg Leu	tac Tyr 5 cgc Arg ggg Gly	aag Lys atc Ile cac His	Thr gag Glu aag	ctg Leu ctg Leu 40	Ala aag Lys 25 gag Glu	Glu 10 ggc Gly tac Tyr	Val atc Ile aac Asn	Lys gac Asp tac Tyr	Phe ttc Phe aac Asn 45	Glu aag Lys 30 agc Ser	Gly 15 gag Glu cac His	Asp gac Asp aac Asn	96
35	atg Met 1 acc Thr ggc Gly gtc Val	gac Asp ctg Leu aac Asn tat Tyr 50	ggc Gly gtg Val atc Ile 35 atc Ile	aac Asn aac Asn 20 ctg Leu atg Met	tac Tyr 5 cgc Arg ggg Gly gcc Ala	aag Lys atc Ile cac His gac Asp	Thr gag Glu aag Lys	Arg Ctg Leu Ctg Leu 40 Cag Gln	Ala aag Lys 25 gag Glu aag Lys	Glu 10 ggc Gly tac Tyr aac Asn	val atc Ile aac Asn ggc Gly	gac Asp tac Tyr atc ile 60	Phe ttc Phe aac Asn 45 aag Lys	Glu aag Lys 30 agc Ser gtg Val	Gly 15 gag Glu cac His aac Asn	Asp gac Asp aac Asn ttc Phe	96
35 40	atg Met 1 acc Thr ggc Gly gtc Val aag Lys 65 tac	gac Asp ctg Leu aac Asn tat Tyr 50 atc Ile	ggc Gly gtg Val atc Ile 35 atc Ile cgc Arg	aac Asn 20 ctg Leu atg Met cac	tac Tyr 5 cgc Arg ggg Gly gcc Ala aac Asn	aag Lys atc Ile cac His gac Asp atc Ile 70	Thr  gag Glu  aag Lys aag Lys 55	Arg ctg Leu ctg Leu 40 cag Gln gac Asp	Ala aag Lys 25 gag Glu aag Lys ggc Gly	Glu 10 ggc Gly tac Tyr aac Asn ggc Gly	Val atc Ile aac Asn ggc Gly gtg Val 75 ccc	Lys gac Asp tac Tyr atc ile 60 cag Gln gtg	Phe ttc Phe aac Asn 45 aag Lys ctc Leu ctg	aag Lys 30 agc Ser gtg Val gcc Ala	Gly 15 gag Glu cac His aac Asn gac Asp	gac Asp aac Asn ttc Phe cac His 80	96 144 192

														gcc Ala			384	4
5					gac Asp												41	1
10					Γ,S1 & 34		mutat	cions	5									
15														ggc Gly			4.8	3
20														gag Glu 30			96	5
20							_						_	cac His		_	144	1
25														aac Asn			192	2
30	atc Ile 65	cgc Arg	cac His	aac Asn	atc Ile	gag Glu 70	gac Asp	ggc Gly	ggc Gly	gtg Val	cag Gln 75	ctc Leu	gcc Ala	gac Asp	cac His	tac Tyr 80	240	Э
35	cag	cag Gln	aac Asn	acc Thr	ccc Pro 85	atc Ile	ggc Gly	gac Asp	ggc Gly	ccc Pro 90	gtg Val	ctg Leu	ctg Leu	ccc Pro	gac Asp 95	aac Asn	288	3
40														aac Asn 110			336	5
40														999 Gly			384	1
45					gag Glu								•				408	3
50								•										
					Γ, S1		muta	tion	ıs ar	nd Me	et ad	lded	@ pc	os. 1	L			
55														gag Glu			48	3

5										ggc Gly							96
·										tac Tyr							144
10										aac Asn							192
15	aag Lys 65	atc Ile	cgc Arg	cac His	aac Asn	atc Ile 70	gag Glu	gac Asp	ggc Gly	ggc Gly	gtg Val 75	cag Gln	ctc Leu	gcc Ala	gac Asp	cac His 80	240
20										ggc Gly 90							288
25										ctg Leu							336
										ttc Phe							384
30					_	gag Glu	_		_								411
35	Thr	Leu 130 1s F2	Gly	Met /163/	Asp	Glu 75G n	Leu 135	Tyr	Lys								411
	Thr Venu SEQ gac	Leu 130 1s F2 ID 1	Gly 2B, V NOS:3	Met 71637 844 8 tac	Asp A,S17 aag	Glu 75G m	Leu 135 mutat	Tyr	Lys	gtg Val 10							411
35 40	Venu SEQ gac Asp 1	Leu 130 1s F2 ID N ggc Gly	Gly  2B, V  NOS:3  aac  Asn	Met V163F 844 8 tac Tyr	Asp A,S17 aag Lys 5 atc	Glu 75G m acc Thr	Leu 135 mutat cgc Arg	Tyr zions gcc Ala aag	Lys gag Glu ggc	Val	Lys	Phe ttc	Glu	Gly	Asp 15 gac	Thr ggc	
35	Thr VenusEQ gac Asp 1 ctg Leu aac	Leu 130 Is F2 ID N ggc Gly gtg Val	Gly 2B, V NOS:3 aac Asn aac Asn	Met V163A 44 8 tac Tyr cgc Arg 20	Asp A,S17 aag Lys 5 atc Ile	Glu 75G m acc Thr gag Glu aag	Leu 135 mutat cgc Arg ctg Leu	Tyr cions gcc Ala aag Lys	Lys gag Glu ggc Gly 25 tac	Val 10 atc	Lys gac Asp	Phe ttc Phe	Glu aag Lys agc	Gly gag Glu 30 cac	Asp 15 gac Asp	Thr ggc Gly gtc	48
35 40	Venu SEQ gac Asp 1 ctg Leu aac Asn	Leu 130 IS F2 ID N ggc Gly gtg Val atc Ile	Gly  2B, Nos:3  aac Asn  aac Asn  ctg Leu 35  atg	Met V1637 44 8 tac Tyr cgc Arg 20 ggg Gly	Asp A,S17 aag Lys 5 atc Ile cac His	Glu 75G m acc Thr gag Glu aag Lys	Leu 135  mutat  cgc Arg  ctg Leu  ctg Leu  cag	Tyr  cions  gcc Ala  aag Lys  gag Glu 40  aag	Lys  gag Glu  ggc Gly 25  tac Tyr	Val 10 atc Ile	Lys gac Asp tac Tyr	Phe ttc Phe aac Asn	Glu aag Lys agc ser 45	Gly gag Glu 30 cac His	Asp 15 gac Asp aac Asn	Thr ggc Gly gtc Val	48

			acc Thr											288
5			agc Ser 100											336
10			atg Met											384
15			gac Asp											408
20			√163 <i>I</i> 346 8		nutat	cions	s and	d Met	ado	ded (	g pos	5. 1		
25			aac Asn											48
20			aac Asn 20											96
30			ctg Leu											144
35	gtc Val		atg Met											192
40			cac His											240
45			aac Asn											288
			ctg Leu 100											336
50			cac His											384
55			atg Met											411

Venus F2B, M153T, V163A, S175G mutations SEQ ID NOS:348 & 349

5					cgc Arg										48
10					ctg Leu										96
15		_		_	ctg Leu						_			_	144
20					cag Gln 55					_	_			_	192
					gac Asp										240
25					ggc Gly										288
30		_	_		tcc Ser	_	_	_		_				_	336
35					ctg Leu					_	_				384
40					tac Tyr 135										408
45		2B, N NOS:3			S17	75G n	nutat	cions	s and	d Met	ado	ded @	pos	3. 1	
50					acc Thr										48
					gag Glu										96
55					aag Lys										144

5	_				gac Asp	_	_	_				_	_			192
					atc Ile 70											240
10					ccc Pro											288
15			_	_	tac Tyr	_		_	_	_		_				336
20					gtc Val											384
25					gag Glu											411
		F1C ID I			s 1-3 3	L17 d	of E	YFP)	with	n pos	3. 1	Met	remo	oved		
30					gag Glu											48
35					gta Val				_		_					96
40					acc Thr			_	_		_	_			_	144
45					ccc Pro											192
					tgc Cys											240
	65				70											
50	cac				tcc Ser											288

	_		gag Glu 115											*			348
5			, F														
10							gag Glu										48
15							gta Val									ggc Gly	96
20							acc Thr										144
							ccc Pro 55									acc Thr	192
25							tgc Cys										240
30							tcc Ser										288
35							gac Asp									gag Glu	336
40			ttc Phe 115			•											351
	YFP	F1C	, F46	5L mi	ıtat:	ion a	and p	oos.	1 Me	et re	emove	ed.					
45	SEQ	ID I	NOS:	356 8	§ 35°	7											
	gtg Val 1	agc Ser	aag Lys	ggc Gly	gag Glu 5	gag Glu	ctg Leu	ttc Phe	acc Thr	999 Gly 10	gtg Val	gtg Val	ccc Pro	atc Ile	ctg Leu 15	gtc Val	48
50							aac Asn										96
55							tac Tyr										144

													acc Thr			192
5								_	_				atg Met	_		240
10													cag Gln			288
15					_	_	_				_	_	gcc Ala 110			336
20	_	ttc Phe														348
				9R mi 358 8												
25													ccc Pro			48
30													gtg Val 30			96
35			_		_	_				_	_	_	aag Lys			144
40													gtg Val			192
													cac His			240
45								_	_		_		gtc Val	_		288
50													cgc Arg 110			336
55		_		gag Glu												351

YFP F1C, K79R mutation and pos. 1 Met removed SEQ ID NOS:360 & 361

5						gag Glu						48	3
10		_	_		_	gta Val		_	_			 96	5
15						acc Thr						144	ł
						ccc Pro						192	3
20						tgc Cys 70			_	_	_	 240	)
25						tcc Ser						288	3
30						gac Asp						336	5
35	_	ttc Phe										348	3
40				5F mi 362 8									
45						gag Glu						48	3
						gac Asp						96	ĵ
50						gcc Ala						144	F
55						ctg Leu						192	3

							gcc Ala							240
5							atg Met							288
10							ggc Gly 105							336
15			gag Glu											351
20			6F mu 364 8		and p	os.1	L Met	rem	nove	i				
25							acc Thr					gtc <sup>.</sup> Val		48
20							cac His 25							96
30							aag Lys							144
35	acc Thr						tgg Trp							192
40							cgc Arg							240
4E							ccc Pro							288
45							aac Asn 105							336
50		gag Glu 115												348
55			9Κ mι 366 δ					•					•	

										ccc Pro		48
5										gtg Val 30		96
10										aag Lys		144
15			Leu							gtg Val		192
20										cac His		240
										gtc Val	gag Glu	288
25										cgc Arg 110		336
30	 _	gag Glu										351
35		9K mi 368 8		and p	os.	1 Me	et re	emove	ed			
40										atc Ile		48
45										tcc Ser 30		96
										ttc Phe		144
50										acc Thr		192
55										atg Met		240

													cag Gln			288
5													gcc Ala 110			336
10			gag Glu 115													348
15				, V68 370 8		muta	ation	ıs					٠			
20													ccc Pro			48
05	_		_	_	 _	_				_		_	gtg Val 30		ggc Gly	96
25													aag Lys			144
30													gtg Val			192
35	ttc Phe 65												cac His			240
40													gtc Val			288
45													cgc Arg 110			336
45		Lys		gag Glu												351
50				, V68		muta	ation	ns ar	nd po	os. 1	L Met	: rem	noved	i		
55													atc Ile			48

						gta Val											96
5						acc Thr											144
10						ccc Pro											192
15						tgc Cys 70											240
20						tcc Ser											288
						gac Asp											336
25			gag Glu 115														348
30						·											-
30			, F46 NOS:3			mutat	cions	5	·					·			÷
35	SEQ atg	ID i	ios : 3	374 8 aag	375 ggc		gag	ctg									48
	sEQ atg Met 1	ID N gtg Val gag	agc ser	aag Lys gac	ggc Gly 5 ggc	gag	gag Glu gta	ctg Leu aac	Phe ggc	Thr 10 cac	Gly aag	Val	Val agc	Pro gtg	Ile 15 tcc	Leu	48 96
35	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly gat	gag Glu gac	gag Glu gta Val	ctg Leu aac Asn	Phe ggc Gly 25 ggc	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val 30	Ile 15 tcc Ser	Leu ggc Gly atc	
35 40 45	sEQ atg Met 1 gtc Val gag Glu tgc	gtg Val gag Glu ggc Gly	agc Ser ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr 40	ggc Gly 25 ggc Gly	Thr 10 cac His aag Lys	aag Lys ctg Leu	Val ttc Phe acc Thr	Val agc ser ctg Leu 45	gtg Val 30 aag Lys	lle 15 tcc ser ctg Leu	Leu ggc Gly atc Ile	96
35 40	sEQ atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser Ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala	gag Glu gta Val acc Thr ccc Pro 55	ctg Leu aac Asn tac Tyr 40 gtg Val	ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	Val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser Ctg Leu acc Thr	Leu ggc Gly atc Ile acc Thr	96 144

5		ttc Phe 100											;	336
		gag Glu											3	351
10		6L,F0		cions	s and	d pos	5. 1	Met	remo	oved				
15		ggc Gly												48
20		ggc Gly 20												96
25		gat Asp										tgc Cys	1	144
-		aag Lys											1	192
30		ctg Leu											2	240
35		ttc Phe											2	288
40		ttc Phe 100											3	336
45	ttc Phe												3	348
		1L mi 378 8			•									
50		aag Lys												48
55		gac Asp 20												96

				ggc Gly													144
5				ggc Gly													192
10				ggc Gly													240
15				ttc Phe													288
20				ttc Phe 100													336
				gag Glu									. •				351
25																	
				1L mi 380 &			and p	os.	1 Me	et re	emove	ed					
30 -				ggc Gly													48
35	Val 1 gag	Ser ctg	Lys		Glu 5 gac	Glugta	Leu aac	Phe ggc	Thr	Gly 10 aag	Val ttc	Val agc	Pro gtg	Ile tcc	Leu 15 ggc	Val gag	48 96
	Val 1 gag Glu	Ser ctg Leu gag	Lys gac Asp	ggc Gly	Glu 5 gac Asp	Glu gta Val acc	Leu aac Asn tac	Phe ggc Gly ggc	Thr cac His 25	Gly 10 aag Lys ctg	Val ttc Phe	val agc ser	Pro gtg Val	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
35 40	Val 1 gag Glu ggc Gly	ctg Leu gag Glu	gac Asp ggc Gly 35	Gly ggc Gly 20 gat	Glu 5 gac Asp gcc Ala	gta Val acc Thr	Leu aac Asn tac Tyr	Phe ggc Gly ggc Gly 40 ccc	Thr cac His 25 aag Lys	Gly 10 aag Lys ctg Leu	ttc Phe acc Thr	agc Ser ctg Leu	gtg Val aag Lys 45	tcc Ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96
35	Val 1 gag Glu ggc Gly acc Thr	ctg Leu gag Glu acc Thr 50	gac Asp ggc Gly 35 ggc Gly	ggc Gly 20 gat Asp	Glu 5 gac Asp gcc Ala ctg Leu	gta Val acc Thr ccc Pro	Leu aac Asn tac Tyr gtg Val 55 ttc	Phe ggc Gly ggc Gly 40 ccc Pro	Thr cac His 25 aag Lys tgg Trp cgc	Gly 10 aag Lys ctg Leu ccc Pro	ttc Phe acc Thr	Val agc ser ctg Leu ctc Leu 60 gac	gtg Val aag Lys 45 gtg Val	tcc Ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ctc Leu	96 144
35 40	Val 1 gag Glu ggc Gly acc Thr ggc Gly 65 cac	ctg Leu gag Glu acc Thr 50 tac Tyr	gac Asp ggc Gly 35 ggc Gly ggc Gly ttc	ggc Gly 20 gat Asp aag Lys	Glu 5 gac Asp gcc Ala ctg Leu cag Gln	gta Val acc Thr ccc Pro tgc Cys 70	Leu aac Asn tac Tyr gtg Val 55 ttc Phe gcc	Phe ggc Gly ggc Cly 40 ccc Pro gcc Ala atg	Thr  cac His 25  aag Lys  tgg Trp  cgc Arg	Gly 10 aag Lys ctg Leu ccc Pro tac Tyr	ttc Phe acc Thr acc Thr	Val agc Ser ctg Leu ctc Leu 60 gac Asp	Pro gtg Val aag Lys 45 gtg Val cac His	tcc Ser 30 ttc Phe acc Thr atg Met	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ctc Leu cgg Arg 80 cgc	96 144 192

		gag Glu 115												348
5			V mut 382 &											
10											ccc Pro			48
15											gtg Val 30			96
20											aag Lys			144
20											gtg Val			192
25											cac His			240
30		_		_		_	_		_		gtc Val	_		288
35											cgc Arg 110			336
40			gag Glu											351
.0						•								
45			5W mi 384 8		and p	os.	1 Me	et re	emove	ed		•		
											atc Ile			48
50		-						_		_	 tcc Ser 30			96
55											ttc Phe			144

				gtg Val 55						192
5				ttc Phe						240
10				gcc Ala						288
15				 gac Asp		_	_	_	 	336
20	ttc Phe									348
	F1C		nutat k 381							
25				gag Glu						48
30 -				gta Val						96
35				acc Thr						144
40				ccc Pro 55						192
.0				tgc Cys						240
45				tcc Ser						288
50				gac Asp						336
55	aag Lys									351

CFP F1C, S65A mutation and pos. 1 Met removed SEQ ID NOS:388 & 389

5	gtg Val 1	agc Ser	aag Lys	ggc Gly	gag Glu 5	gag Glu	ctg Leu	ttc Phe	acc Thr	999 Gly 10	gtg Val	gtg Val	ccc Pro	atc Ile	ctg Leu 15	gtc Val		48
10										aag Lys								96
15										ctg Leu								144
										ccc Pro								192
20										tac Tyr								240
25										gaa Glu 90								288
30										tac Tyr								336
35		ttc Phe																348
									•									
40		F1C,					572A	muta	ation	ns								
45										acc Thr 10								.48
										cac His								96
50										aag Lys								144
55										tgg Trp							•	192

				cag Gln 70										2	40
5				aag Lys											88
10				aag Lys										3	36
15		gag Glu												3	51
20			₹66W, \$ 393	, S72 3	2A mi	ıtati	ions	and	pos	. 1 N	Met 1	remov	ved		
25				gag Glu											48
				gta Val											96
30				acc Thr										1	44
35				ccc Pro										1	92
40				tgc Cys 70										2	40
45				tcc Ser										2	88
70				gac Asp										. 3	36
50	ttc Phe													3.	48

CFP F1C, F64L,S65T,Y66W mutations SEQ ID NOS:394 & 395

5												ccc Pro		ctg Leu		48
10												gtg Val 30				96
15												aag Lys			1	44
												gtg Val			1	92
20												cac His			2	240
25												gtc Val			2	88
30				_	_	_				_		cgc Arg 110	-		3	336
35	aag Lys														3	351
40	F1C,				muta	ation	ns ar	nd po	os. 1	L Met	: rem	noved	i			
45												atc Ile				48
												tcc Ser 30				96
50	 -	_	_				_	_		_	_	ttc Phe		_	1	44
55												acc Thr			1	.92

					tgc Cys 70						240
5					tcc Ser						288
10					gac Asp						336
15		ttc Phe							·		348
20		F1C,									
25					gag Glu						48
30					gac Asp						96
					gcc Ala						144
35				_	ctg Leu						192
40					cag Gln 70						240
45					aag Lys						288
50					aag Lys						336
	_	aag Lys									351

BFP F1C, Y66H mutation and pos. 1 Met removed SEQ ID NOS:400 & 401

5							ttc Phe								48
10							ggc Gly								96
15			 _	_			ggc Gly 40	_	_		_	_		_	144
							ccc Pro								192
20						Phe	gcc Ala								240
25							atg Met								288
30				_	_	_	ggc Gly			_		-	_	 _	336
35	_	ttc Phe	 												348
40		F1C				ions	5								
45							ctg Leu								48
							aac Asn								96
50			 	_	_		tac Tyr 40		_	_		_	_		144
55							gtg Val								192

					ttc Phe								240
5					gcc Ala								288
10					gac Asp								336
15	 _	ttc Phe 115	 										351
٠											٠		
20		, F64 NOS:4		ions	s and	d pos	3. 1	Met	remo	oved			
25					ttc Phe								48
30					ggc Gly								96
50					ggc Gly 40								144
35					ccc Pro								192
40					gcc Ala								240
45					atg Met								288
50					ggc Gly								336
50		gag Glu 115											348

(YFP F2C corresponds to aa residues 118-239 of YFP) SEQ ID NOS:406 & 407 atg gac acc ctg gtg aac cqc atc qaq ctq aaq qqc atc qac ttc aaq Met Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys 10 gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac aac agc 96 10 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser 2.0 cac aac gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg 144 His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val 15 35 aac ttc aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc 192 Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala 55 20 gac cac tac cag cag aac acc ccc atc ggc gac ggc ccc gtg ctg 240 Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu 25 ccc gac aac cac tac ctg agc tac cag tcc gcc ctg agc aaa gac ccc 288 Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro 90 aac gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc gcc gcc 336 30 Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala 100 105 110 ggg atc act ctc ggc atg gac gag ctg tac aag 369 Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys 35 115 YFP F2C, Y203F mutation 40 SEQ ID NOS:408 & 409 gac acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag qaq 48 Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu 10 45 gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac aac agc cac 96 Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His 20 50 aac gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg aac 144 Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn 35 ttc aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc gac 192 55 Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp 50

YFP F2C, with Met added @ pos. 1

									ggc Gly 75					240
5									ctg Leu					288
10									ttc Phe					336
15			_	_	gag Glu	_		_						366
							٠						,	
20			nutat k 41:		and	Met	adde	ed @	pos.	. 1				
25									aag Lys					48
30									gag Glu					96
				-	_	_	_	_	aag Lys			_		144
35									ggc Gly					192
40									gac Asp 75					240
45									gcc Ala					288
50									gag Glu					336
30								tac Tyr						369

## YFP F2C, Y203H mutation SEQ ID NOS:412 & 413

5	acc Thr											48
10	ggc Gly											96
15	gtc Val											144
	aag Lys 50											192
20	tac Tyr											240
25	aac Asn											288
30	aag Lys	-	_	_	_	_				_	_	 336
35	act Thr											366
40	F2C,			and	Met	adde	ed @	pos.	. 1			
45	gac Asp											48
	gac Asp											96
50	aac Asn											144
55	ttc Phe 50											192

								ccc Pro									240
5								cac His									288
10								gtc Val									336
15								gag Glu 120									369
20	(CFI	P F20	, N14 C COI NOS:4	resp	onds	s to	aa 1	resio	lues	118-	-239	of \	(FP)				
25								gag Glu									48
30								aag Lys									96
35								aag Lys 40									144
								gag Glu									192
40								atc Ile									240
45								cag Gln									288
50		_	-	-			_	ctg Leu	_					_	_		336
55								ctg Leu 120			·						366

SEO ID NOS:418 & 419 atg gac acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag 48 Met Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys gag gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac atc agc 96 Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Ile Ser 10 cac aac gtc tat atc atg gcc gac aag cag aag aac ggc atc aag gtg 144 His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys Val 40 15 aac ttc aag atc cgc cac aac atc qaq qac qqc aqc qtq caq ctc qcc 192 Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala 55 20 gac cac tac cag cag aac acc ccc atc ggc gac ggc ccc gtg ctg 240 Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu 70 ccc gac aac cac tac ctg agc acc cag tcc gcc ctg agc aaa gac ccc 288 25 Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro 85 aac gag aag cgc gat cac atg gtc ctg ctg gag ttc gtg acc gcc gcc 336 Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala Ala 30 100 ggg atc act ctc ggc atg gac gag ctg tac aag 369 Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys 115 35 CFP F2C, M153T mutation 40 SEQ ID NOS:420 & 421 gac acc ctg gtg aac cgc atc gag ctg aag ggc atc gac ttc aag gag 48 Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu 45

gac ggc aac atc ctg ggg cac aag ctg gag tac aac tac aac agc cac

Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn Ser His

aac gtc tat atc acc gcc gac aag cag aag aac ggc atc aag gtg aac

Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn Gly Ile Lys Val Asn

ttc aag atc cgc cac aac atc gag gac ggc agc gtg cag ctc gcc gac

Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu Ala Asp

40

55

20

35

50

50

55

CFP F2C, N146I mutation and Met added @ pos. 1

96

144

		_		acc Thr 70				_			 _	_		240
5				agc Ser										288
10				atg Met										336
15				gac Asp										366
							٠						·	
20			muta 423	ation 3	n and	l Met	ado	ded @	og 🤋	3. 1				
25				aac Asn										48
30				ctg Leu										96
				acc Thr										144
35				cac His										192
40				aac Asn 70										240
45				ctg Leu										288
50	aac Asn			cac His										336
				atg Met							٠			369

CFP F2C, N146I,M153T mutations SEQ ID NOS:424 & 425

5								aag Lys 10							48
10	_			_			_	gag Glu					_		96
15								aag Lys							144
								ggc Gly							192
20								gac Asp						ccc Pro 80	240
25								gcc Ala 90							288
30								gag Glu							336
35		act Thr													366
40		F2C,			ıtati	ons	and	Met	adde	ed @	pos	. 1			
45								ctg Leu 10							48
								ctg Leu							96
50								cag Gln							144
55								gac Asp							192

						atc Ile						240
5			-	_		cag Gln	_	_	_	_		288
10						ctg Leu 105						336
15						ctg Leu						369
00					•							
20	F2C											
25						ctg Leu						48
30						ctg Leu 25						96
35						cag Gln						144
00						gac Asp						192
40						ggc Gly						240
45						tcc Ser						288
50						ctg Leu 105						336
55						tac Tyr	•					366

	F2C				and	Met	adde	ed @	pos	. 1	•			
5	gac Asp													48
10	 gac Asp						_	_					_	96
15	aac Asn													144
15	ttc Phe 50													192
20	cac His												ctg Leu 80	240
25	gac Asp													288
30	gag Glu													336
35	atc Ile													369
40	F2C,				atio	ons								
45	acc Thr													48
45	ggc Gly													96
50	gtc Val		_	_	_	_	_	_			_	_		144
55	aag Lys 50													192

												ctg Leu			240
5												gac Asp			288
10												gcc Ala 110			336
15				atg Met											366
20				/163/ k 435	atio	ons a	and N	Met a	addeo	1 @ p	os.	1			
25	_	_	_		_			_	_			gac Asp	_		48
30												tac Tyr 30		•	96
												atc Ile			144
35												cag Gln			192
40												gtg Val			240
45												aaa Lys			288
50	aac Asn											acc Thr 110			336
				ggc Gly											369

CFP F2C, M153T, V163A mutations SEQ ID NOS:436 & 437

5		acc Thr															48
10		ggc Gly															96
15		gtc Val															144
		aag Lys 50															192
20		tac Tyr					Pro									ccc Pro 80	240
25		aac Asn															288
30		aag Lys															336
35		act Thr															366
40		F2C,					ıtati	ons.	and	Met	adde	ed @	pos.	. 1			
45	atg Met 1	gac Asp	acc Thr	ctg Leu	gtg Val 5	aac Asn	cgc Arg	atc Ile	gag Glu	ctg Leu 10	aag Lys	ggc Gly	atc Ile	gac Asp	ttc Phe 15	aag Lys	48
		gac Asp															96
50		aac Asn															144
55		ttc Phe 50															192

					aac Asn 70										÷	240
5		_			ctg Leu	_		_		_	_	_		_		288
10					cac His											336
15					atg Met			_		_						369
	•							÷								
20				11535 k 445	Γ,and	1 V16	53A r	nutat	ions	5						
25					cgc Arg											48
30					ggg Gly											96
00					gcc Ala											144
35					aac Asn											192
40					acc Thr 70				_				_	_		240
45					agc Ser											288
50	gag Glu				atg Met											336
50					gac Asp											366

		F2C					and V	/163 <i>I</i>	A mut	tatio	ons a	and I	Met a	adde	1 @ p	pos.	1	
5		gac Asp																48
10		gac Asp																96
15		aac Asn																144
		ttc Phe 50																192
20		cac His																240
25		gac Asp																288
30		gag Glu																336
35		atc Ile																369
40	(CGI	P F2( FP F2 ID N	C CC	orres	spond	ds to			es 1.	18-23	39 of	E YFI	?)					
45		acc Thr	-	-		_			_	_			_		_			48
50		ggc Gly																96
JU		gtc Val				_	_	-	_	_				_				144
55		aag Lys 50																192

5		cag Gln											240
		cac His			_		_	_	_		_		288
10		cgc Arg											336
15		ctc Leu 115									•		366
20		C, Mi NOS:4		ıtat:	ions	and	Met	adde	ed @	pos	. 1		
25		acc Thr											48
30		ggc Gly											96
35		gtc Val 35											144
		aag Lys											192
40		tac Tyr											240
45		aac Asn											288
50		aag Lys											336
55		act Thr 115											369

CGFP F2C, V163A,T203Y mutations SEQ ID NOS:448 & 449

5	gac Asp 1	acc Thr	ctg Leu	gtg Val	aac Asn 5	cgc Arg	atc Ile	gag Glu	ctg Leu	aag Lys 10	ggc Gly	atc Ile	gac Asp	ttc Phe	aag Lys 15	gag Glu	48
10								aag Lys									96
15								aag Lys 40									144
								gag Glu									192
20	cac His 65	tac Tyr	cag Gln	cag Gln	aac Asn	acc Thr 70	Pro	atc Ile	ggc Gly	gac Asp	ggc Gly 75	ccc Pro	gtg Val	ctg Leu	ctg Leu	ccc Pro 80	240
25								cag Gln									288
30								ctg Leu									336
35								ctg Leu 120									366
40		P F20					ıtati	ons	and	Met	adde	ed @	pos.	. 1			
45								atc Ile									48
	gag Glu	gac Asp	ggc Gly	aac Asn 20	atc Ile	ctg Leu	ggg Gly	cac His	aag Lys 25	ctg Leu	gag Glu	tac Tyr	aac Asn	tac Tyr 30	aac Asn	agc Ser	. 96
50								gac Asp 40									144
55								atc Ile									192

										ggc Gly				240
5	-			_	_		_		_	ctg Leu	_	_		288
10										ttc Phe				336
15				atg Met	_		_		_					369
20	P F20			BT,ar	nd T2	203Y	muta	ation	ıs					
25										atc Ile				48
30										aac Asn				96
										ggc Gly				144
35										gtg Val 60				192
40										ccc Pro				240
45										agc Ser				288
50										gtg Val				336
			_	gac Asp		_		_						366

	P F20			nd Ti	203Y	muta	ation	ns ai	nd Me	et ad	ded	@ pc	os. 1	
5	gac Asp													48
10	gac Asp													96
15	aac Asn													144
	ttc Phe 50													192
20	cac His													240
25	gac Asp													288
30 .	gag Glu													336
35	atc Ile													369
40	P F20			nd T2	203Y	muta	ation	ıs						
45	acc Thr													48
.0	ggc Gly		_		_	_						_		96
50	gtc Val													144
55	aag Lys 50													192

					acc Thr 70											240
5					agc Ser											288
10					atg Met											336
15					gac Asp											366
		·														
20			C, Mi Nos:4			nd T2	203Y	muta	ation	ns ar	nd Me	et ad	dded	@ pc	os. 1	
25					aac Asn											48
30					ctg Leu											96
					acc Thr											144
35					cac His											192
40					aac Asn 70											240
45					ctg Leu											288
50	aac Asn		_	_	cac His	_	_	_	_					_	_	336
JU					atg Met											369

CGFP F2C, N146I,M153T,V163A,and T203Y mutations SEQ ID NOS:460 & 461

5	acc Thr														48
10	ggc Gly														96
15	gtc Val		_	_	_	_	_				_	_			144
	aag Lys 50														192
20	tac Tyr	_					_				_	_	ccc Pro 80		240
25	aac Asn														288
30	aag Lys														336
35	act Thr														366
40	P F20			63A,	and	T203	3Υ mι	ıtati	ions	and	Met	adde	ed @ p	os.	1
45	 gac Asp	 _			_					_	_	_	_		48
	gac Asp														96
50	aac Asn														144
55	ttc Phe 50														192

										ggc Gly						40
5										tcc Ser 90					2	88
10										ctg Leu					3	36
15										tac Tyr						69
	•							•								
20	(BF	F2C, P F20 ID 1	COI	cresp	ponds	s to	aa 1	resio	dues	118-	-239	of Y	(FP)			
25										aag Lys 10						48
30										gag Glu						96
35										aag Lys					1	44
										ggc Gly					1	92
40										gac Asp					2:	40
45										gcc Ala 90					2	88
50	gag Glu									gag Glu					3	36
55						gac Asp										66

		ID I					and	Mec	auu	eu w	pos	. 1				
5		gac Asp													aag Lys	48
10		gac Asp														96
15		aac Asn														144
		ttc Phe 50														192
20		cac His														240
25		gac Asp														288
30		gag Glu														336
35		atc Ile														369
40	(Ver	us F2 nus E ID N	72C (	corre	espor	nds t		a res	sidue	es 13	L8-23	39 of	E YFI	?)		
45		acc Thr														48
		ggc Gly														96
50		gtc Val														144
55		aag Lys 50														192

BFP F2C, Y145F mutation and Met added @ pos. 1

												ctg Leu			240
5												gac Asp			288
10												gcc Ala 110			336
15			_	gac Asp		_		_					•		366
20	ıs F2 ID 1			tatio l	on ar	nd Me	et ad	dded	@ pa	os. I	l.				
25												gac Asp			48
30												tac Tyr 30			96
												atc Ile			144
35												cag Gln			192
40												gtg Val			240
45												aaa Lys			288
50												acc Thr 110			336
				atg Met											369

Venus F2C, M153T, S175G mutations SEQ ID NOS:472 & 473

5					cgc Arg											48
10	_			_	ggg Gly		_	-						_		96
15					gcc Ala											144
					aac Asn											192
20					acc Thr 70											240
25					agc Ser											288
30					atg Met											336
35					gac Asp											366
00		ıs F2 ID N			L75G 5	muta	ation	ns ar	nd Me	et ad	ded	@ po	os. 1	L		
40					aac Asn	_			_	_			_		_	48
45					ctg Leu											96
50					acc Thr											144
00					cac His											192
55					aac Asn 70											240

5			aac Asn														288
			aag Lys													gcc Ala	336
10			act Thr 115														369
15			2C, 7 NOS:				muta	ation	ıs								
20			ctg Leu														48
25			aac Asn														96
			tat Tyr 35														144
30	ttc Phe	aag Lys 50	atc Ile	cgc Arg	cac His	aac Asn	atc Ile 55	gag Glu	gac Asp	ggc Gly	ggc Gly	gtg Val 60	cag Gln	ctc Leu	gcc Ala	gac Asp	192
35			cag Gln														240
40			cac His														288
45			cgc Arg														336
			ctc Leu 115								•						366
50			2C, V 10S:4				nutat	ions	and	1 Met	: add	ded @	) pos	s. 1			
55			acc Thr														48

										ctg Leu							96
5										cag Gln							144
10										gac Asp							192
15										ggc Gly							240
20										tcc Ser 90							288
										ctg Leu						gcc Ala	336
25										tac Tyr							369
30					Γ,V16 \$ 481		and §	31750	3 mut	atio	ons						
30 35	SEQ	ID Nacc	10S:4	180 8 gtg	x 481	l cgc	atc	gag	ctg	aag Lys 10	ggc.						48
	gac Asp 1	acc Thr	NOS:4 ctg Leu aac	gtg Val atc	aac Asn 5	cgc Arg	atc Ile cac	gag Glu aag	ctg Leu ctg	aag Lys	ggc Gly tac	Ile aac	Asp	Phe aac	Lys 15 agc	Glu	<b>48</b> 96
35 40	gac Asp 1 gac Asp	acc Thr ggc Gly	ctg Leu aac Asn	gtg Val atc Ile 20	aac Asn 5 ctg Leu	cgc Arg ggg Gly	atc Ile cac His	gag Glu aag Lys	ctg Leu ctg Leu 25	aag Lys 10 gag	ggc Gly tac Tyr	Ile aac Asn	Asp tac Tyr	Phe aac Asn 30	Lys 15 agc Ser	Glu cac His	
35	gac Asp 1 gac Asp aac Asn	acc Thr ggc Gly gtc Val	ctg Leu aac Asn tat Tyr 35	gtg Val atc Ile 20 atc Ile	aac Asn 5 ctg Leu acc Thr	cgc Arg ggg Gly gcc Ala	atc Ile cac His gac Asp	gag Glu aag Lys aag Lys 40	ctg Leu ctg Leu 25 cag Gln	aag Lys 10 gag Glu	ggc Gly tac Tyr aac Asn	aac Asn ggc Gly	Asp tac Tyr atc Ile 45	Phe aac Asn 30 aag Lys	Lys 15 agc ser gcc Ala	Glu cac His aac Asn	96
35 40	gac Asp 1 gac Asp aac Asn ttc Phe	acc Thr ggc Gly gtc Val aag Lys 50 tac	ctg Leu aac Asn tat Tyr 35 atc Ile	gtg Val atc Ile 20 atc Ile cgc Arg	aac Asn 5 Ctg Leu acc Thr	cgc Arg ggg Gly gcc Ala aac Asn	atc Ile cac His gac Asp atc Ile 55	gag Glu aag Lys aag Lys 40 gag Glu	ctg Leu ctg Leu 25 cag Gln gac Asp	aag Lys 10 gag Glu aag Lys	ggc Gly tac Tyr aac Asn ggc Gly	aac Asn ggc Gly gtg Val 60 ccc	Asp tac Tyr atc Ile 45 cag Gln	Phe aac Asn 30 aag Lys ctc Leu ctg	Lys 15 agc ser gcc Ala gcc Ala	Glu cac His aac Asn gac Asp	96 1 <sub>.</sub> 44

				gat Asp 100														336
5				ggc Gly														366
10				M1537			, and	S175	5G mi	utat:	ions	and	Met	adde	ed @	pos.	1	
15				ctg Leu														48
20				aac Asn 20														96
25				tat Tyr														144
				atc Ile														192
30				cag Gln														240
35				cac His														288
40				cgc Arg 100			_	_	_	_					_	_		336
45				ctc Leu														369
	(YFI	P F1I	X fi	oosit cagme	ent c	corre				a res	sidue	es 1-	-158	of Y	(FP)			
50				ggc Gly														48
55				ggc Gly 20														96

						acc Thr				144
5						acc Thr				192
10						ccc Pro 75				240
15						ggc Gly				288
20						aag Lys				336
						atc Ile				384
25						cac His				432
30						gac Asp 155				471
35		79R r 186 8								
40		Lys			Phe	999 Gly				48
45						aag Lys				96
<b>70</b>				Thr		ctg Leu				144
50						ccc Pro				192
55						tac Tyr 75				240

		gac Asp											288
5		atc Ile											336
10		ttc Phe 115											384
15		ttc Phe		Asp									432
20		aac Asn											474
25		X, K' NOS:4			, pos	3. 1	Met	remo	oved	·			
		aag Lys											48
30		gac Asp											96
35		ggc Gly 35											144
40		ggc Gly											192
45		ggc Gly											240
10		ttc Phe											288
50		ttc Phe											336
55		gag Glu 115											384

											gag Glu			432
5		_		_		_	_	gac Asp 155	_	_				471
10														
	F1D													
15											ccc Pro			48
20											gtg Val 30			96
25	 		 _	_			_	_		_	aag Lys			144
30											gtg Val			192
00											cac His		:	240
35		_		_	-	_		_			gtc Val	_	 :	288
40											cgc Arg 110			336
45											ctg Leu		:	384
50											ctg Leu			432
00								gcc Ala 155						474

YFP F1DX, Y66F mutation, pos. 1 Met removed SEQ ID NOS:492 & 493

5		ggc Gly								48
10		ggc Gly 20							:	96
15		gat Asp							1.	44
		aag Lys							19	92
20		ctg Leu							. 24	40
25		ttc Phe							28	88
30		ttc Phe 100							3:	36
35		ggc Gly							38	84
		gag Glu							43	32
40		cac His							4	71
45										
		 59K m								
50		aag Lys							4	48
55		gac Asp 20							9	96

					gcc Ala									144
5	_			_	ctg Leu									192
10					aag Lys 70									240
15					aag Lys									288
20					aag Lys									336
					gac Asp							Leu	ggc	384
25			_		gac Asp				_		_	_		432
30					aac Asn 150									474
35			59K r 196 8		cion,	pos	3. 1	Met	remo	oved				
40				Glu	gag Glu			Thr						48
45					gta Val									96
					acc Thr									144
50					ccc Pro									192
55					tgc Cys 70									240

					tcc Ser								288
5					gac Asp								336
10					acc Thr								384
15					ggc Gly								432
20					gtc Val 150								471
25			F1DX		Q6 9 <b>N</b>	1 mut	atio	ons		·	٠.		
					gag Glu								48
30					gac Asp	-			_	_			96
35		Gly			gcc Ala								144
40	Cys				ctg Leu	Pro							192
45					atg Met 70								240
					aag Lys								288
50					aag Lys								336
55					gac Asp								384

					gac Asp										43:	2
5					aac Asn 150										47	4
10														÷		
			X, V6 500 8		Q6 9 <b>1</b> 1	1 mut	catio	ons,	and	pos	. 1 1	let i	remov	red		
15					gag Glu										4:	8
20					gta Val										91	6
25					acc Thr										14	4
30					ccc Pro										19:	2
					tgc Cys 70										24	0
35					tcc Ser										288	8
40					gac Asp									_	 330	6
45					acc Thr										384	4
50				_	ggc Gly			_			_	_			432	2
		_			gtc Val 150			_	-	_	_	_			47	1

CFP F1DX, F64L mutation SEQ ID NOS:502 & 503

5				gag Glu										48
10				gac Asp										96
15				gcc Ala										144
10				ctg Leu										192
20				cag Gln 70									aag Lys 80	240
25				aag Lys										288
30				aag Lys						_	_	_		336
35				gac Asp										384
				gac Asp										432
40				aac Asn 150										474
45									*					
			54L n	ion,	, pos	s. 1	Met	remo	oved					
50	 _	_		 gag Glu	_							_	-	48
55				gta Val										96

									acc Thr				144
5		 _	_						acc Thr			_	192
10									ccc Pro 75				240
15									ggc Gly				288
20									aag Lys				336
20			_		_			_	atc Ile	 _	_	 atc Ile	384
25									cac His				432
30									gac Asp 155				471
35		54L, 506 &		Γ, Υ6 7	56W r	nutat	cions	5					
40									gly ggg				48
45									aag Lys				96
									ctg Leu				144
50									ccc Pro				192
55									tac Tyr 75				240

				aag Lys											288
5				aag Lys										gag Glu	336
10				gac Asp											384
15				gac Asp											432
20				aac Asn 150											474
25		X, FONOS:		, Y66V 9	√ mut	atio	ons,	and	pos	. 1 N	Met 1	cemor	red .		
				gag Glu											48
30				gta Val											96
35				acc Thr											144
40				ccc Pro											192
45				tgc Cys 70											240
40				tcc Ser											288
50				gac Asp	_				_		_	_			336
55				acc Thr											384

					aac Asn 135							432
5		_		_	tat Tyr	_	_	_	_	_		471
10												
15			56W t 510 t									
					gag Glu							48
20					gta Val							96
25					acc Thr							144
30					ccc Pro 55							192
35					tgc Cys							240
					tcc Ser							288
40					gac Asp							336
45					acc Thr							384
50					ggc Gly 135							432
55					gtc Val							474

SEQ ID NOS:512 & 513 gtg agc aag ggc gag gtg ttc acc ggg gtg gtg ccc atc ctg gtc 48 Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val 10 15 gag ctg gac ggc gac gta aac ggc cac aag ttc agc gtg tcc ggc gag 96 Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu 10 ggc gag ggc gat gcc acc tac ggc aag ctg acc ctg aag ttc atc tgc 144 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys 15 acc acc ggc aag ctg ccc gtg ccc tgg ccc acc ctc gtg acc acc ttc 192 Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe 55 60 20 ggc tgg ggc ctg cag tgc ttc gcc cqc tac ccc gac cac atg aag cgg 240 Gly Trp Gly Leu Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys Arg 70 . 75 cac gac ttc ttc aag tcc gcc atg ccc gaa ggc tac gtc cag gag cgc 288 25 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg 85 acc atc ttc ttc aag gac gac ggc aac tac aag acc cgc gcc gag gtg 336 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val 30 . 100 105 aag ttc gag ggc gac acc ctg gtg aac cgc atc gag ctg aag ggc atc 384 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile 115 35 gac ttc aag gag gac ggc aac atc ctq qqq cac aaq ctq gaq tac aac 432 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn 135 40 tac aac agc cac aac qtc tat atc atq qcc qac aaq caq 471 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln 145 150 155 45 CFP F1DX, Y66W, N146I mutations SEQ ID NOS:514 & 515 50 atg gtg agc aag ggc gag gtg ttc acc ggg gtg gtg ccc atc ctg 48 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu 15 gte gag etg gae gge gae gta aac gge cac aag tte age gtg tee gge 96 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly

CFP F1DX, Y66W mutation, and pos. 1 Met removed

						gcc Ala											:	144
5						ctg Leu												192
10						cag Gln 70											2	240
15						aag Lys											2	288
20						aag Lys												336
						gac Asp										ggc Gly	3	384
25						gac Asp											4	132
30						aac Asn 150								cag			4	174
35	•																	
00			(, Y6 10s:5			[ mut	atio	ons,	pos.	. 1 N	Met r	remov	red					
40	SEQ gtg	ID N	10S:5	516 8 ggc Gly	\$ 517 gag		ctg	ttc	acc	999	gtg	gtg	ccc					48
40	SEQ gtg Val 1	agc Ser	aag Lys gac	ggc Gly ggc	gag Glu 5 gac	7 gag	ctg Leu aac	ttc Phe ggc	acc Thr	999 Gly 10 aag	gtg Val ttc	gtg Val agc	ccc Pro	Ile tcc	Leu 15 ggc	Val gag		48
	gtg Val 1 gag Glu	agc ser ctg Leu gag Glu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr cac His 25	999 Gly 10 aag Lys	gtg Val ttc Phe	gtg Val agc Ser	ccc Pro gtg Val	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	1	
40	gtg Val 1 gag Glu ggc Gly	agc Ser ctg Leu gag Glu acc	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	ctg Leu aac Asn tac Tyr	ttc Phe ggc Gly ggc Gly 40	acc Thr cac His 25 aag Lys	ggg Gly 10 aag Lys ctg Leu	gtg Val ttc Phe acc Thr	gtg Val agc ser ctg Leu	ccc Pro gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	Val gag Glu tgc Cys		<sub>.</sub> 96

					tcc Ser			Pro						288
5					gac Asp									336
10					acc Thr									384
15					ggc Gly									432
20					gtc Val 150									471
25		F1D ID I			3T mi 9	ıtat	ions				. •	٠.		
					gag Glu									48
30					gac Asp									96
35					gcc Ala									144
40	_			_	ctg Leu									192
<b>4</b> 5			 	_	cag Gln 70	_			_		-	_		240
40					aag Lys									288
50					aag Lys									336
55					gac Asp									384

							ggc Gly 135										4:	32
5							gtc Val										4	74
10																		
				56W,1 520 8			tatio	ons,	pos	. 1 1	Met 1	remo	red					
15							ctg Leu										4	48
20							aac Asn										9	96
25							tac Tyr										14	14
30							gtg Val 55										19	92
							ttc Phe										24	10
35							gcc Ala										28	38
40	acc Thr	atc Ile	ttc Phe	ttc Phe 100	aag Lys	gac Asp	gac Asp	ggc Gly	aac Asn 105	tac Tyr	aag Lys	acc Thr	cgc Arg	gcc Ala 110	gag Glu	gtg Val	33	36
45							ctg Leu										38	34
50	gac Asp						aac Asn 135										43	32
			_			-	tat Tyr			_	_	_	_				47	71

· CFP F1DX, N146I mutation SEQ ID NOS:522 & 523

5				gag Glu											8
10				gac Asp										9	96
15				gcc Ala										14	: <b>4</b>
				ctg Leu										19	12
20			_	cag Gln 70	_		_	-			_	_	_	. 24	0
25				aag Lys										28	8
30				aag Lys										33	6
35				gac Asp										38	4
				gac Asp										43	.2
40				aac Asn 150										47	4
45															
	F1DX ID N			atior	ı, po	os. 1	L Met	ren	noved	i					
50				gag Glu										4	8
55				gta Val										9	6

						acc Thr							144
5						ccc Pro							192
10						tgc Cys 70							240
15						tcc Ser							288
20					_	gac Asp	_		_	_	_	 	336
20						acc Thr					Lys	atc Ile	384
25						ggc Gly							432
30						gtc Val 150							471
35	CED	יחום	z M:	ובית	muta	ation			-				
33		ID 1				atior 7	1						
40						gag Glu							48
45						gac Asp							,96
		Gly				gcc Ala							144
50						ctg Leu							192
55						cag Gln 70							240

	cgg Arg	cac His	gac Asp	ttc Phe	ttc Phe 85	aag Lys	tcc Ser	gcc Ala	atg Met	ccc Pro 90	gaa Glu	ggc Gly	tac Tyr	gtc Val	cag Gln 95	gag Glu	288
5						aag Lys											336
10	gtg Val	aag Lys	ttc Phe 115	gag Glu	ggc Gly	gac Asp	acc Thr	ctg Leu 120	gtg Val	aac Asn	cgc Arg	atc Ile	gag Glu 125	ctg Leu	aag Lys	ggc Gly	384
15						gac Asp											432
20						aac Asn 150											474
25				153T 528 &		ation 9	1, po	os. 3	l Met	rer	nove	i	٠		٠.		
	gtg Val 1	agc Ser	aag Lys	ggc Gly	gag Glu 5	gag Glu	ctg Leu	ttc Phe	acc Thr	999 Gly 10	gtg Val	gtg Val	ccc Pro	atc Ile	ctg Leu 15	gtc Val	48
30						gta Val											96
35						acc Thr											144.
40					_	ccc Pro											192
45						tgc Cys 70											240
						tcc Ser											288
50						gac Asp											336
55						acc Thr											384

								Gly 999						432
5		_		_				gcc Ala	_	_	_			471
10														
		K, N: NOS:5			nutat	cions	3							
15								acc Thr 10						48
20								cac His						96
25	_	 	 _	_				aag Lys	_		_	_		144
30								tgg Trp						192
00								cgc Arg						240
35								ccc Pro 90						288
40								aac Asn						336
45								aac Asn						384
50			 	_				ctg Leu			_	_		432
								acc Thr						474

CFP F1DX, N146I,M153T mutations, pos. 1 Met removed SEQ ID NOS:532 & 533

5			gag Glu									48
10			gta Val									96
15			acc Thr									144
			ccc Pro									192
20			tgc Cys 70								cgg Arg 80	240
25			tcc Ser									288
30			gac Asp									336
35			acc Thr									384
			ggc Gly			_		_	_			432
40			gtc Val 150									471
45		56W, 534 8	5I,M1 5	L53T	muta	atior	ıs					
50			gag Glu									48
			gac Asp									96
55			gcc Ala									144

5						ctg Leu											192
v						cag Gln 70				Arg							240.
10						aag Lys											288
15						aag Lys											336
20						gac Asp						Ile					384
25						gac Asp											432
						aac Asn 150											474
30 .		F1D ID I				5I,M: 7	L53T	muta	ation	ns, a	and p	os.	1 Me	et re	emove	ed	
30	SEQ gtg	ID 1	10S:	536 8 ggc	≩ 535 gag		ctg	ttc	acc	999	gtg	gtg	ccc	atc	ctg	gtc	48
	SEQ gtg Val 1	agc Ser	aag Lys gac	ggc Gly ggc	gag Glu 5 gac	7 gag	ctg Leu aac	ttc Phe ggc	acc Thr	999 Gly 10 aag	gtg Val ttc	gtg Val	ccc Pro	atc Ile tcc	ctg Leu 15	gtc Val gag	48 96
35	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr cac His 25	999 Gly 10 aag Lys	gtg Val ttc Phe	gtg Val agc Ser	ccc Pro gtg Val	atc Ile tcc Ser 30	ctg Leu 15 ggc Gly	gtc Val gag Glu tgc	
35 40	gtg Val 1 gag Glu ggc Gly	agc Ser ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	ctg Leu aac Asn tac Tyr	ttc Phe ggc Gly ggc Gly 40 ccc	acc Thr cac His 25 aag Lys	ggg Gly 10 aag Lys ctg Leu	gtg Val ttc Phe acc Thr	gtg Val agc Ser ctg Leu	ccc Pro gtg Val aag Lys 45	atc Ile tcc Ser 30 ttc Phe	ctg Leu 15 ggc Gly atc Ile	gtc Val gag Glu tgc Cys	96
35 40	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser ctg Leu gag Glu acc Thr 50 tgg	aag Lys gac Asp ggc Gly 35 ggc	ggc Gly ggc Gly 20 gat Asp aag Lys ctg	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr gtg Val 55	ttc Phe ggc Gly ggc Gly 40 ccc Pro	acc Thr cac His 25 aag Lys tgg Trp	ggg Gly 10 aag Lys ctg Leu ccc Pro	gtg Val ttc Phe acc Thr	gtg Val agc Ser ctg Leu ctc Leu 60	ccc Pro gtg Val aag Lys 45 gtg Val	atc Ile tcc Ser 30 ttc Phe acc Thr	ctg Leu 15 ggc Gly atc Ile acc Thr	gtc Val gag Glu tgc Cys ttc Phe	96

						aac Asn 105						336
5						aac Asn						384
10						ctg Leu						432
15		_		_		acc Thr	_	_	_	_		471
20			55A r 538 8									
25						ttc Phe						48
20						ggc Gly 25						96
30						ggc Gly						144
35						ccc Pro						192
40						gcc Ala						240
45						atg Met						288
43						ggc Gly 105						336
50						gtg Val						384
55						atc Ile						432

							gtc Val									474
5					muta & 54:		, pos	s.1 M	Met :	remo	ved					
10							ctg Leu									48
15							aac Asn									96
20							tac Tyr	Gly								144
20							gtg Val 55							ttc Phe		192
25							ttc Phe								•	240
30							gcc Ala									288
35							gac Asp									336
40							ctg Leu									384
40							aac Asn 135									432
45							tat Tyr									471
50					Y66V x 543		72A n	nutat	cions	5						
55	atg	gtg	agc	aag	ggc	gag	gag Glu									48

	-	gag Glu	_	_		_	_				_		_			96
5		ggc Gly														144
10	_	acc Thr 50			_	_										192
15		gcc Ala														240
20		cac His														288
		acc Thr													gag Glu	336
25		aag Lys														384
30		gac Asp 130														432
35		tac Tyr														474
40		F1DX					2A mi	ıtat:	ions	, pos	s. 1	Met	remo	oved		
45		agc Ser														48
50	gag Glu	ctg Leu	_		_	_				_		_			 	96
<b>55</b>		gag Glu		_	_				_	_			_		tgc Cys	144
55		acc Thr 50														192

5	gcc Ala 65	tgg Trp	ggc	ctg Leu	cag Gln	tgc Cys 70	ttc Phe	gcc Ala	cgc Arg	tac Tyr	ccc Pro 75	gac Asp	cac His	atg Met	aag Lys	cgg Arg 80	240
	cac His	gac Asp	ttc Phe	ttc Phe	aag Lys 85	tcc Ser	gcc Ala	atg Met	ccc Pro	gaa Glu 90	ggc Gly	tac Tyr	gtc Val	cag Gln	gag Glu 95	cgc Arg	288
10				ttc Phe 100													336
15				ggc Gly													384
20				gag Glu													432
25				cac His													471
				56Η π 546 &													
30				aag Lys													48
35				gac Asp 20													96
40				ggc													144
45				ggc Gly													192
				ggc Gly													240
50				ttc Phe													288
55				ttc Phe 100													336

														ctg Leu		384
5														ctg Leu		432
10						aac Asn 150										474
15				56H r 548 8		tion,	, pos	3. 1	Met	remo	oved					
20														atc Ile		48
20	_	_	_		_	_				_		_	_	tcc Ser 30	 	96
25									_	_		_	_	ttc Phe	_	144
30														acc Thr	ttc Phe	192
35	ggc Gly 65													atg Met		240
40														cag Gln		288
10														gcc Ala 110		336
45														aag Lys		384
50														gag Glu		432
55						gtc Val 150										471

BFP F1DX, F64L,Y66H mutations SEQ ID NOS:550 & 551

5				gag Glu								ctg Leu	48
10				gta Val									96
15				acc Thr									144
				ccc Pro 55									192
20				tgc Cys									240
25				tcc Ser									288
30 .				gac Asp									336
35				acc Thr									384
				ggc Gly 135									432
40				gtc Val									474
45													
		54L,\ 552 8		ation	ıs, p	os.	1 Me	et re	emove	ed			
50				ctg Leu									48
55				aac Asn									96

			acc Thr										144
5			ccc Pro										192
10			tgc Cys 70									:	240
15			tcc Ser									:	288
20			gac Asp									:	336
			acc Thr							Lys			384
25			ggc Gly									•	432
30			gtc Val 150									•	471
35		54L, 554 8	H, Y1 5	L45F	muta	ation	ns	•					
40			gag Glu										48
45			gac Asp										96
.0	Gly		gcc Ala	Thr								:	144
50			ctg Leu								acc Thr		192
55			cag Gln 70									. 2	240

					aag Lys											2	288
5					aag Lys												336
10					gac Asp											3	384
15	_		-		gac Asp				_			_	-	_		4	132
20					aac Asn 150												174
25		X, Fe			H, Y:	L45F	muta	ation	ns ar	nd po	os. I	L Met	ren	nove	i		
					gag Glu												48
30					gta Val												96
35					acc Thr											1	144
40					ccc Pro											1	192
45					tgc Cys 70											2	240
40					tcc Ser											2	288
50				_	gac Asp	_				_		_	_			3	336
55					acc Thr											3	384

										gag Glu			432
5								gac Asp 155					471
10													
15			muta § 559	ation 9	ı								
										ccc Pro			48
20										gtg Val 30			96
25										aag Lys			144
30										gtg Val			192
35										cac His			240
00										gtc Val			288
40				_	_	_			_	cgc Arg 110	_		336
45										ctg Leu			384
50	atc Ile									ctg Leu		,	432
55								gcc Ala 155					474

BFP F1DX, Y145F mutation and pos. 1 Met removed SEQ ID NOS:560 & 561

5				gag Glu									48
10				gta Val									96
15				acc Thr									144
20				ccc Pro									192
				tgc Cys 70									240
25			_	tcc Ser	_	_		_		_	_	 _	288
30				gac Asp									336
35				acc Thr									384
40				ggc Gly									432
.0				gtc Val 150								٠	471
45													
	F1DX ID N			Y145	SF mu	ıtati	lons						
50				gag Glu									48
55				gac Asp									96

	 		 _	gcc Ala				_	_		_	_	_		144
5				ctg Leu											192
10				cag Gln 70											240
15				aag Lys											288
20				aag Lys											336
				gac Asp								Leu		ggc Gly	384
25				gac Asp				_				-			432
30				aac Asn 150											474
35		(, F4 10s:5		, Y145 5	5F mu	utati	lons,	and	d pos	3. 1	Met	remo	oved		
40		Lys	Glu	gag Glu			Thr								48
45				gta Val											<sub>.</sub> 96
	Glu			acc Thr											144
50			 ata	CCC	ata	ccc	taa	ccc	acc	ctc	gtg	acc	acc	cta	192
				Pro								Thr			

	cac His	gac Asp	ttc Phe	ttc Phe	aag Lys 85	tcc Ser	gcc Ala	atg Met	ccc Pro	gaa Glu 90	ggc Gly	tac Tyr	gtc Val	cag Gln	gag Glu 95	cgc Arg	288
5	acc Thr	atc Ile	ttc Phe	ttc Phe 100	aag Lys	gac Asp	gac Asp	ggc	aac Asn 105	tac Tyr	aag Lys	acc Thr	cgc Arg	gcc Ala 110	gag Glu	gtg Val	336
10	aag Lys	ttc Phe	gag Glu 115	ggc Gly	gac Asp	acc Thr	ctg Leu	gtg Val 120	aac Asn	cgc Arg	atc Ile	gag Glu	ctg Leu 125	aag Lys	ggc Gly	atc Ile	384
15	gac Asp	ttc Phe 130	aag Lys	gag Glu	gac Asp	ggc Gly	aac Asn 135	atc Ile	ctg Leu	ggg Gly	cac His	aag Lys 140	ctg Leu	gag Glu	tac Tyr	aac Asn	432
20	ttc Phe 145	aac Asn	agc Ser	cac His	aac Asn	gtc Val 150	tat Tyr	atc Ile	atg Met	gcc Ala	gac Asp 155	aag Lys	cag Gln				471
25		us Fi ID 1				tatio 7	on .										
30	atg Met 1	gtg Val	agc Ser	aag Lys	ggc Gly 5	gag Glu	gaģ Glu	ctg Leu	ttc Phe	acc Thr 10	Gly ggg	gtg Val	gtg Val	ccc Pro	atc Ile 15	ctg Leu	48
00	gtc Val	gag Glu	ctg Leu	gac Asp 20	ggc Gly	gac Asp	gta Val	aac Asn	ggc Gly 25	cac His	aag Lys	ttc Phe	agc Ser	gtg Val 30	tcc Ser	ggc Gly	96
35							acc Thr										144
40							ccc Pro 55										192
45	ttc Phe 65	ggc Gly	tac Tyr	ggc Gly	ctg Leu	cag Gln 70	tgc Cys	ttc Phe	gcc Ala	cgc Arg	tac Tyr 75	ccc Pro	gac Asp	cac His	atg Met	aag Lys 80	240
50	cgg Arg	cac His	gac Asp	ttc Phe	ttc Phe 85	aag Lys	tcc Ser	gcc Ala	atg Met	ccc Pro 90	gaa Glu	ggc Gly	tac Tyr	gtc Val	cag Gln 95	gag Glu	288
- •	cgc Arg	acc Thr	atc Ile	ttc Phe 100	ttc Phe	aag Lys	gac Asp	gac Asp	ggc Gly 105	aac Asn	tac Tyr	aag Lys	acc Thr	cgc Arg 110	gcc Ala	gag Glu	336
55	gtg Val	aag Lys	ttc Phe 115	gag Glu	ggc Gly	gac Asp	acc Thr	ctg Leu 120	gtg Val	aac Asn	cgc Arg	atc Ile	gag Glu 125	ctg Leu	aag Lys	ggc Gly	384

5						gac Asp										432
						aac Asn 150										474
10																
15		ıs F: ID N				atio	on, p	pos.	1 Me	et re	emove	ed				
						gag Glu										48
20		_	_		_	gta Val				_		_			 	96
25						acc Thr										144
30						ccc Pro										192
35						tgc Cys 70										240
		_			_	tcc Ser	_	_		_			_	_	 _	288
40	_	-		_		gac Asp					_					336
45						acc Thr										384
50	gac Asp					ggc Gly										432
55						gtc Val 150										471

Venus F1DX, F46L,F64L mutations
SEQ ID NOS:570 & 571

	1		-	-10	5	0.0	Olu	200	2 110	10	O. J	vai		15	Deu	•	
10		gag Glu															96
15		ggc Gly															144
		acc Thr 50															192
20		ggc Gly															240
25		cac His															288
30		acc Thr															336
35		aag Lys															384
		gac Asp 130															432
40		tac Tyr															474
45																	
		ıs F1 ID N					ıtati	.ons,	pos	s. 1	Met	remo	oved				
50		agc Ser															48
55		ctg Leu															96

atg gtg agc aag ggc gag gag ctg ttc acc ggg gtg gtg ccc atc ctg Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu

		gat Asp											144
5		aag Lys											192
10		ctg Leu											240
15		ttc Phe											288
20		ttc Phe 100											336
20		ggc Gly									Lys	atc Ile	384
25		gag Glu	-				_		_	-			432
30		cac His											471
35		F64I 574 8			nutat	cions	5						
40		aag Lys											48
45		gac Asp 20											96
	Gly	ggc Gly		-				 _		_	-		144
50		ggc Gly											192
55		ggc Gly											240

				aag Lys											288
5				aag Lys										gag Glu	336
10				gac Asp											384
15				gac Asp											432
20				aac Asn 150											474
25	FP F: ID 1			53T r 7	nutat	ions	s, ai	nd po	os. I	L Met	rer	nove	i		
30				gag Glu											48
00				gta Val											96
35				acc Thr											144
40				ccc Pro											192
45		 _	_	tgc Cys 70		_	_			_		_	_		240
				tcc Ser											288
50			_	gac Asp	_				_		_	_			336
55				acc Thr											384

5	_		_		_				_			_		gag Glu		432
J						gtc Val 150										471
10																
				F64L 578 8		3T mu 9	utati	ions								
15														ccc Pro		48
20														gtg Val 30		96
25					_	_				_	_		_	aag Lys		144
30														gtg Val		192
														cac His		240
35														gtc Val		288
40														cgc Arg 110		336
45														ctg Leu		384
50		-		_		_				-			_	ctg Leu		432
				_		aac Asn 150	_				_	-	_	_		474

. Venus F1DX, F64L, M153T mutations, pos. 1 Met removed SEQ ID NOS:580 & 581

5											atc Ile	gtc Val	48
10	 _	_	 _	_				_	_		tcc Ser 30	 	96
15											ttc Phe		144
											acc Thr		192
20											atg Met		240
25											cag Gln		288
30											gcc Ala 110		336
35											aag Lys		384
											gag Glu		432
40								gcc Ala	 _	_			471
45													
	ıs F1 ID N				M153	3T mi	ıtati	ions					
50											ccc Pro		48
55											gtg Val 30		96

			 ggc Gly	_	_				-	_		_	_	_		144
5	_		.ggc Gly	_	_											192
10			ggc Gly													240
15			ttc Phe													288
20			ttc Phe 100													336
			gag Glu												ggc Gly	384
25		_	aag Lys		_				_			_	_			432
30			agc Ser			-				_	_	_	_			474
35			F46I 584 8			153T	muta	ation	ns, a	and p	pos.	1 M€	et re	emove	ed	
40			ggc Gly													48
45			ggc Gly 20													96
			gat Asp													144
50			aag Lys													192
55			 ctg Leu	_			_	_			_		_	_		240

						tcc Ser		Pro							288
5					_	gac Asp	_			_		_	_	 	336
10						acc Thr									384
15						ggc Gly									432
20						gtc Val 150									471
25	(YF	F2DX, P F2I ID 1	OX co	orres	spond	@ po ds to 7		idues	s 159	9-239	of	YFP)	)		
30						aag Lys									48
						ctc Leu									96
35						ctg Leu									144
40						gac Asp									192
45						gcc Ala 70									240
		aag Lys								·					246
50		F2DX ID N				atior	1					٠.			
55	aag	aac	ggc	atc	aag	gtg Val									48

5		agc Ser												96
		ggc Gly			_		_				_	_	_	144
10		ctg Leu 50												192
15		ttc Phe												240
20	aag Lys						-							243
25		F2DX ID N				ı, ar	nd Me	et ad	dded	@ pc	os. I	L		
		aag Lys												48
30		ggc Gly								_	_			96
35		gac Asp												144
40		gcc Ala 50												192
45		gag Glu												240
	tac Tyr	_												246
50		F2DX ID N	•											
55		aac Asn												48

				gcc Ala									96
5	_			ctg Leu		_				_	_	_	144
10				ccc Pro									192
15				gcc Ala 70									240
	aag Lys												243
20			203H 594 8	ation 5	n,and	d Met	ado	ded (	pos	3. 1			
25				aag Lys									48
30 -				ctc Leu									96
35				ctg Leu									144
33				gac Asp									192
40				gcc Ala 70									240
45	tac Tyr	aag Lys											246
50			203T 596 8	atior 7	1						÷		
				gtg Val									48
55				gcc Ala									96

5														cag Gln		144
														ctg Leu		192
10	-				_	_						_	_	 ctg Leu		240
15	aag Lys															243
20				03T n 598 8			ano	d Met	ado	ded (	) pos	s. 1				
25														atc Ile 15		48
25														ccc Pro		96
30														act Thr		144
35														gtc Val		192
40														gag Glu		240
	tac Tyr	-														246
45	CED	FODX	7 171	.63A,	Vana	T mi	ıtati	One								
	SEQ						icacı	Ons								
50														gag Glu 15		48
55														atc Ile		96

				gtg Val	_	_		_				_	_		_		144
5				aaa Lys													192
10				acc Thr												:	240
15	aag Lys			•		•										:	243
20				163A, 502 8			ıtat:	ions	and	i Met	ado	ded @	pos	s. 1			
_0				ggc Gly													48
25				gtg Val 20													96
30 -				ccc Pro												:	144
35				agc Ser												:	192
40				gtg Val												2	240
40	tac Tyr	_													•	:	246
45																	
			•	S175			ion										
50				ggc Gly													48
55				gtg Val 20													96

		ccc Pro										144
5		agc Ser										192
10		gtg Val										240
15	aag Lys											246
20		S175		ion,	and	d Met	ado	ded (	pos	3. 1		
		ggc Gly					_		_			 48
25		gtg Val 20	-	_	_			-	_			96
30		ccc Pro										144
35		agc Ser										192
40		gtg Val										240
-10	aag Lys										·	246
45												
		V163	-	mut	atio	ons						
50		atc Ile										48
55		cag Gln 20										96

						ctg Leu											144
5						ccc Pro											192
10						gcc Ala 70											240
15	aag Lys																243
20					3A, 8 2 611	61 <b>7</b> 50	3 mut	atio	ons,	and	Met	adde	ed @	pos	. 1		
	_	_				aag Lys	_			_		_					48
25						ctc Leu											96
30						ctg Leu											144
35						gac Asp				-	_	_		_	_	_	192
40						gcc Ala 70											240
40		aag Lys															246
45	(YFI	P F1I	CO	resp		Met s to			dues	1-15	59 of	f YFI	?)				
50			_			gag Glu	_								_	-	48
55						gta Val											96

				acc Thr							144
5				ccc Pro							192
10				tgc Cys 70							240
15				tcc Ser							288
20			_	gac Asp	_		_	_	_	 	336
				acc Thr							384
25				ggc Gly							432
30				gtc Val 150							474
35		9R mu 514 8									
40				gag Glu							48
45				gac Asp							96
.0				gcc Ala							144
50				ctg Leu							192
55				cag Gln 70							240

											cag Gln 95		288
5											gcc Ala		336
10											aag Lys		384
15											gag Glu		432
20	Tyr				tat Tyr								477
25			utat: k 61	Met	@ pa	os. :	l rem	nove	i				
											ctg Leu 15		48
30											ggc Gly		96
35											atc Ile		144
40											acc Thr		192
45											aag Lys		240
70											gag Glu 95		288
50											gag Glu		336
55											ggc Gly		384

		_	 _		aac Asn 135		_			_	_			4	32
5		_		_	tat Tyr		_	_	_	_	_	_		. 4	74
10															
	F1D ID I														
15					gag Glu									•	48
20					gta Val									!	96
25					acc Thr									14	44
30					ccc Pro 55									19	92
					tgc Cys									24	40
35					tcc Ser									28	88
40					gac Asp									33	36
45			 	_	acc Thr	_			_			_	_	 38	34
50					ggc Gly 135									43	32
					gtc Val									.47	77

YFP F1D, Y66F mutation, Met @ pos. 1 removed SEQ ID NOS:620 & 621

5				gag Glu									48
10				gta Val									96
15				acc Thr									144
				ccc Pro									192
20				tgc Cys 70									240
25				tcc Ser									288
30				gac Asp									336
35				acc Thr									384
				ggc Gly									432
40		_		gtc Val 150		_	_	_	_	_	_		474
45													
	YFP SEQ		9K mι 522 δ										
50				gag Glu									48
55				gac Asp									96

					gcc Ala											144
5					ctg Leu											192
10				_	aag Lys 70	_		_	_			_		_	_	240
15					aag Lys											288
20	_				aag Lys	_	_				_		_	_		336
					gac Asp											384
25		_	_		gac Asp				_			-	_			432
30					aac Asn 150											477
35			9K mi 524 8		ion,	Met	@ pc	os. 3	l rem	nove	i					
40				_	gag Glu											48
45					gta Val											96
50					acc Thr											144
					ccc Pro											192
55			 _	-	tgc Cys 70		_				_		_	_		240

			tcc Ser								288
5			gac Asp								336
10			acc Thr								384
15			ggc Gly								432
20	Asn		gtc Val 150								474
25	rine ID 1		Q6 9M 7	muta	ation	ıs					
			gag Glu								48
30			gac Asp								96
35			gcc Ala								144
40			ctg Leu								192
45			atg Met 70								240
			aag Lys								288
50			aag Lys								336
55			gac Asp								384

		ttc Phe												432
5		aac Asn		_			_	_	_	_	_	_		477
10														
		F1D NOS:		nutat	cions	3, Me	et @	pos	.1 re	emove	ed			
15		aag Lys												48
20		gac Asp												96
25		ggc Gly 35												144
30		ggc Gly												192
		ggc Gly												240
35		ttc Phe												288
40		ttc Phe												336
45		gag Glu 115												384
50		aag Lys												432
		agc Ser												474

## CFP F1D, F64L mutation SEQ ID NOS:630 & 631

5											ccc Pro		48
10											gtg Val 30		96
15											aag Lys		144
											gtg Val		192
20											cac His		240
25		_		_		_	_		_		gtc Val	_	 288
30											cgc Arg 110		336
35											ctg Leu		384
											ctg Leu		432
40											cag Gln		477
45										•			
	CFP SEQ				Met	@ pc	os. 1	l rem	noved	i			
50											atc Ile		48
55											tcc Ser 30		96

			acc Thr							144
5			ccc Pro							192
10			tgc Cys 70							240
15			tcc Ser							288
20			gac Asp							336
			acc Thr						atc Ile	384
25			ggc Gly							432
30			gtc Val 150							474
35		5L mi 534 8								
40		Lys	gag Glu		Phe					48
45			gac Asp							96
-10			gcc Ala							144
50			ctg Leu							192
55			cag Gln 70							240

	cgg Arg	cac His	gac Asp	ttc Phe	ttc Phe 85	aag Lys	tcc Ser	gcc Ala	atg Met	ccc Pro 90	gaa Glu	ggc Gly	tac Tyr	gtc Val	cag Gln 95	gag Glu	288
5						aag Lys											336
10						gac Asp											384
15						gac Asp											432
20	aac Asn 145	tac Tyr	aac Asn	agc Ser	cac His	aac Asn 150	gtc Val	tat Tyr	atc Ile	atg Met	gcc Ala 155	gac Asp	aag Lys	cag Gln	aag Lys		477
25			, F46 NOS:6			ion, 7	Met	@ po	os. :	l ren	noved	i					
30						gag Glu											48
00						gta Val											96
35						acc Thr											144
40						ccc Pro											192
45						tgc Cys 70											240
	cac His					tcc Ser											288
50	acc Thr	atc Ile	ttc Phe	ttc Phe 100	aag Lys	gac Asp	gac Asp	ggc Gly	aac Asn 105	tac Tyr	aag Lys	acc Thr	cgc Arg	gcc Ala 110	gag Glu	gtg Val	336
55						acc Thr											384

5				aac Asn 135						432
				tat Tyr						474
10										
			F64L ⊊ 639	ation	ıs					
15				gag Glu						48
20				gta Val						96
25				acc Thr						144
30				ccc Pro 55						192
				tgc Cys						240
35				tcc Ser						288
40				gac Asp						336
45				acc Thr						384
50				ggc Gly 135						432
				gtc Val						477

YFP F1D, F46L, F64L mutations, Met @ pos. 1 removed SEQ ID NOS:640 & 641

5					gag Glu									48
10					gta Val									96
15					acc Thr									144
					ccc Pro									192
20					tgc Cys 70									240
25					tcc Ser									288
30				_	gac Asp	_			_		_	_	 	336
35					acc Thr									384
55					ggc Gly			 		_	_			432
40					gtc Val 150								-	474
45			F64L, 542 8		3Τ mι 3	ıtati	ions							
50					gag Glu									48
					gac Asp	-			_		_			96
55	gag Glu				gcc Ala			 _	_		_	-		144

5						ctg Leu											192
						cag Gln 70											240 <sup>.</sup>
10						aag Lys											288
15						aag Lys											336
20						gac Asp						Ile					384
25						gac Asp											432
						aac Asn 150											477
30				F64L,		3T mu 5	ıtati	ions,	. Met	e p	pos.	1 re	emove	ed			
35	SEQ gtg	ID Nagc	NOS:	544 8 ggc	& 645 gag		ctg	ttc	acc	<b>a</b> gg	gtg	gtg	ccc	atc			48
	SEQ gtg Val 1	agc Ser	aag Lys gac	ggc Gly ggc	gag Glu 5 gac	gag	ctg Leu aac	ttc Phe ggc	acc Thr	ggg Gly 10	gtg Val	gtg Val agc	ccc Pro	atc Ile tcc	Leu 15 ggc	Val gag	48
35	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr cac His 25	ggg Gly 10 aag Lys	gtg Val ttc Phe	gtg Val agc Ser	ccc Pro gtg Val	atc Ile tcc Ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
35	gtg Val 1 gag Glu ggc Gly	agc Ser ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	ctg Leu aac Asn tac Tyr	ttc Phe ggc Gly ggc Gly 40 ccc	acc Thr cac His 25 aag Lys	ggg Gly 10 aag Lys ctg Leu	gtg Val ttc Phe acc Thr	gtg Val agc Ser ctg Leu	ccc Pro gtg Val aag Lys 45	atc Ile tcc Ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96
35	gtg Val 1 gag Glu ggc Gly acc Thr	agc ser ctg Leu gag Glu acc Thr 50 tac	aag Lys gac Asp ggc Gly 35 ggc	ggc Gly ggc Gly 20 gat Asp aag Lys ctg	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr gtg Val 55	ttc Phe ggc Gly ggc Gly 40 ccc Pro	acc Thr cac His 25 aag Lys tgg Trp	ggg Gly 10 aag Lys ctg Leu ccc Pro	gtg Val ttc Phe acc Thr	gtg Val agc Ser ctg Leu ctc Leu 60	ccc Pro gtg Val aag Lys 45 gtg Val	atc Ile tcc Ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag glu tgc Cys ctg Leu	96 144

				gac Asp								336
5				acc Thr								384
10				ggc Gly							,	432
15				gtc Val 150								474
		 		 _								
20			F46L, 546 (	31 mu 7	ıtati	ions						
25				gag Glu						ctg Leu		48
				gac Asp								96
30				gcc Ala								144
35	tgc Cys			ctg Leu							:	192
40				cag Gln 70							:	240
45				aag Lys							:	288
				aag Lys								336
50				gac Asp							:	384
55				gac Asp								432

			cac His											477
5								•						
			,M15:		ıtat:	ions	, Me	t@j	pos.	1 re	emove	ed .		
10			gag Glu 5											48
15			gac Asp											96
20			gcc Ala							Leu				144
25			ctg Leu											192
			cag Gln											240
30			aag Lys 85											288
35			aag Lys											336
40			gac Asp											384
45			gac Asp											432
70		_	aac Asn	_				_	_	_	-	_		474
50														
			, F64I & 651		33T n	nutat	ions	3						
55			ggc Gly 5											48

5	_	 _	_	 _	gta Val				_		_				96
					acc Thr										144
10					ccc Pro 55										192
15					tgc Cys										240
20					tcc Ser										288
25					gac Asp									gag Glu	336
					acc Thr										384
30					ggc Gly 135										432
35					gtc Val										477
40			F46L,		53T n	nutat	ions	s, Me	et @	pos.	. 1 1	remov	red		
45					ctg Leu										.48
					aac Asn										96
50					tac Tyr										144
55					gtg Val 55									ctg Leu	192

						tgc Cys 70												240
5						tcc Ser												288
10						gac Asp												336
15						acc Thr												384
20						ggc Gly												432
						gtc Val 150												474
25																	٠	
		F1D				L, NI	146I	muta	ation	ıs.								
	OLQ	10 1	100.0	754 (	. 05.	,								*				
30 -	atg	gtg	agc	aag	ggc	gag Glu												48
30	atg Met 1	gtg Val gag	agc Ser	aag Lys gac	ggc Gly 5	gag	Glu gta	Leu	Phe ggc	Thr 10 cac	Gly aag	Val ttc	Val agc	Pro gtg	Ile 15 tcc	Leu ggc		48 96
	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly	gag Glu gac	Glu gta Val acc	Leu aac Asn	Phe ggc Gly 25 ggc	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val 30	Ile 15 tcc ser	ggc Gly		
35 40	atg Met 1 gtc Val gag Glu	gtg Val gag Glu ggc Gly acc	agc Ser Ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp	gta Val acc Thr	aac Asn tac Tyr 40	ggc Gly 25 ggc Gly	Thr 10 cac His aag Lys	aag Lys ctg Leu	ttc Phe acc Thr	agc ser ctg Leu 45	gtg Val 30 aag Lys	Ile 15 tcc ser ctg Leu	Leu ggc Gly atc Ile		96
35	atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser Ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala	gta Val acc Thr ccc Pro 55	Leu aac Asn tac Tyr 40 gtg Val	ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	aag Lys ctg Leu ccc Pro	ttc Phe acc Thr acc Thr 60	Val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser ctg Leu acc Thr	ggc Gly atc Ile acc Thr		96 144
35 40	atg Met 1 gtc Val gag Glu tgc Cys ctg Leu 65	gtg Val gag Glu ggc Gly acc Thr 50 ggc Gly	agc Ser ctg Leu gag Glu 35 acc Thr tac Tyr	aag Lys gac Asp 20 ggc Gly ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys ctg Leu	gag Glu gac Asp gcc Ala ctg Leu cag Gln	gta Val acc Thr ccc Pro 55 tgc Cys	Leu aac Asn tac Tyr 40 gtg Val ttc Phe	ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp cgc Arg	Gly aag Lys ctg Leu ccc Pro tac Tyr 75 gaa	ttc Phe acc Thr acc Thr 60 ccc Pro	Val agc Ser ctg Leu 45 ctc Leu gac Asp	gtg Val 30 aag Lys gtg Val cac	Ile 15 tcc Ser ctg Leu acc Thr	ggc Gly atc Ile acc Thr aag Lys 80 gag		96 144 192

					gac Asp											384
5					gac Asp											432
10					aac Asn 150											477
15		, F40 NOS:0			N1463 7	I mut	atio	ons,	Met	@ po	os. :	l rem	nove	i		
20					gag Glu											48
20					gta Val											96
25					acc Thr											144
30					ccc Pro											192
35			_	_	tgc Cys 70		_	_			_		_	_		240
40					tcc Ser											288
					gac Asp											336
45					acc Thr											384
50					ggc Gly											432
55					gtc Val 150											474

CFP F1D, F64L,S65T,Y66W mutations SEQ ID NOS:658 & 659

5	gtg Val													48
10	gag Glu													96
15	ggc Gly												1	44
	acc Thr 50												1	92
20	acc Thr			Cys									2	40
25	cac His												2	88
30	acc Thr												3	36
35	aag Lys												3	84
	gac Asp 130	_	 _				_			_	_		4	32
40	tac Tyr	_					_	_	_	_	_	-	4	77
45														
	F1D,			5₩ mi	ıtat:	ions	, Met	z @ p	pos.	1 re	emove	ed		
50	agc Ser													48
55	ctg Leu													96

	 	 _	_	acc Thr		 _	_		_	_		-	144
5				ccc Pro								ctg Leu	192
10				tgc Cys 70									240
15				tcc Ser									288
20			_	gac Asp	_			_		_	_	 	336
				acc Thr									384
25				ggc Gly									432
30				gtc Val 150									474
35		5W mi 562 8											
40				gag Glu									48
45				gac Asp									.96
				gcc Ala									144
50				ctg Leu									192
55				cag Gln 70									240

						aag Lys											288
5						aag Lys											336
10						gac Asp											384
15						gac Asp											432
20						aac Asn 150											477
25				5W mi 564 8		ion,	Met	@ pc	os. I	l ren	noved	i					
30						gag Glu											48
00						gta Val											96
35				_	_	acc Thr			_	_		_	_			_	144
40						ccc Pro											192
45						tgc Cys 70											240
																	288
E0		_			_	tcc Ser	_	_		_			_	_		_	200
50	His	Asp	Phe ttc	Phe ttc	Lys 85 aag		Ala	Met	Pro aac	Glu 90 tac	Gly	Tyr acc	Val	Gln	Glu 95 gag	Arg	336

5										gag Glu		432
ŭ			cac									474
10												
			5W,N:			ation	ıs					
15										ccc Pro		48
20										gtg Val 30		96
25										aag Lys		144
30	_			_	_					gtg Val		192
00										cac His		240
35										gtc Val		288
40										cgc Arg 110		336
45										ctg Leu		384
50										ctg Leu		432
										cag Gln		477

CFP F1D, Y66W,N146I mutations, Met @ pos. 1 removed SEQ ID NOS:668 & 669

5						ctg Leu							gtc Val	48
10	 _	_		_	_	aac Asn			_	_				96
15	 		_	_		tac Tyr		_	_	_	_		_	144
						gtg Val 55								192
20						ttc Phe								240
25						gcc Ala								288
30						gac Asp								336
35						ctg Leu								384
						aac Asn 135								432
40						tat Tyr								474
45														
	F1D,					ation	ıs							
50						gag Glu								48
55						gta Val								96

		 	 _	gcc Ala	Thr			_	_		_	_		144
5			_	ctg Leu										192
10				cag Gln 70										240
15				aag Lys										288
20				aag Lys		Asp								336
				gac Asp										384
25				gac Asp				_			_	_		432
30				aac Asn 150										477
35		, Y66 NOS:6		muta 3	ation	ns, M	Met @	pos	5. 1	remo	oved			
40				gag Glu										48
45				gta Val										96
70				acc Thr										144
	1	35				10								
50	acc	ggc		ccc Pro		CCC								192

					tcc Ser										288
5					gac Asp										336
10					acc Thr										384
15					ggc Gly										432
20					gtc Val 150										474
25			16I r 574 8								,		٠.		
					gag Glu										48
30					gac Asp										96
35					gcc Ala										144
40					ctg Leu										192
45				_	cag Gln 70	_		_	_		_		_	_	240
40					aag Lys										288
50	_				aag Lys	_	_			_		_	_		336
55					gac Asp										384

											ctg Leu		432
5											cag Gln		477
10													
		F1D			, Met	: @ r	pos.	1 re	emove	ed			
15											atc Ile		48
20											tcc Ser 30		96
25											ttc Phe		144
30											acc Thr		192
00											atg Met		240
35											cag Gln		288
40											gcc Ala 110		336
45											aag Lys		384
50	gac Asp										gag Glu		432
					tat Tyr								474

CFP F1D, M153T mutation SEQ ID NOS:678 & 679

5			gag Glu											48
10			gac Asp											96
15			gcc Ala										:	144
			ctg Leu										;	192
20			cag Gln 70											240
25			aag Lys		_	_		-			-	_		288
30 -			aag Lys										;	336
35			gac Asp										;	384
		_	 gac Asp				_			_	_		4	432
40		_	aac Asn 150	_				_	_	_	_	_	4	477
45														
		53T n	ion,	Met	: @ <u>r</u>	os.	1 re	emove	ed					
50			gag Glu											48
55			gta Val											96

						acc Thr											144
5						ccc Pro											192
10						tgc Cys 70											240
15						tcc Ser											288
20	acc Thr	atc Ile	ttc Phe	ttc Phe 100	aag Lys	gac Asp	gac Asp	ggc Gly	aac Asn 105	tac Tyr	aag Lys	acc Thr	cgc Arg	gcc Ala 110	gag Glu	gtg Val	336
						acc Thr											384
25						ggc Gly											432
30						gtc Val 150											474
35				16I,N 582 8		Γ mut	atio	ons									
40	atg Met 1	gtg Val	agc Ser	aag Lys	ggc Gly 5	gag Glu	gag Glu	ctg Leu	ttc Phe	acc Thr 10	ggg Gly	gtg Val	gtg Val	ccc Pro	atc Ile 15	ctg Leu	48
45						gac Asp											96
50	gag Glu					gcc Ala											144
	tgc Cys	acc Thr 50	acc Thr	ggc Gly	aag Lys	ctg Leu	ccc Pro 55	gtg Val	ccc Pro	tgg Trp	ccc Pro	acc Thr 60	ctc Leu	gtg Val	acc Thr	acc Thr	192
55	ttc Phe 65	ggc Gly	tac Tyr	ggc Gly	ctg Leu	cag Gln 70	tgc Cys	ttc Phe	gcc Ala	cgc Arg	tac Tyr 75	ccc Pro	gac Asp	cac His	atg Met	aag Lys 80	240

5					aag Lys											288
					aag Lys											336
10		_			gac Asp		-			_			_	_		384
15					gac Asp											432
20			_		aac Asn 150	_				_	_	_	_	_		477
25		F1D,			r mut	catio	ons,	Met	@ pa	os. 1	L rem	nove	i .			
·					gag Glu											48
30					gta Val				_		_					96
35					acc Thr											144
40				_	ccc Pro											192
45					tgc Cys 70											240
-10					tcc Ser											288
50					gac Asp											336
55	_		 	_	acc Thr	_			_			_	_			384

					aac Asn 135									432
5		_		_	tat Tyr			_	_	_	_	_		474
10			5W, 1		153T	muta	ation	ns						
15					gag Glu									48
20					gta Val									96
25					acc Thr								atc Ile	144
					ccc Pro 55									192
30					tgc Cys									240
35					tcc Ser									288
40					gac Asp									336
45					acc Thr									3,84
					ggc Gly 135									432
50					gtc Val									477

CFP F1D, Y66W, N146I,M153T mutations, Met @ pos. 1 removed SEQ ID NOS:688 & 689

5			ggc Gly		ctg Leu						48
					aac Asn					•	96
10					tac Tyr						144
15					gtg Val 55						192
20					ttc Phe						240
25					gcc Ala						288
					gac Asp						336
30					ctg Leu						384
35					aac Asn 135						432
40					tat Tyr						474
45		, S65 10S:6									
					gag Glu						48
50					gta Val						96
55					acc Thr						144

						ctg Leu											192	2
5		_			_	cag Gln 70				_			-				240	0
10						aag Lys											288	8
15	_					aag Lys		_				_					336	6
20						gac Asp											384	4
20						gac Asp										tac Tyr	432	2
25						aac Asn 150											477	7
30		F1D ID I				ion,	Met	@ po	os. :	l rem	nove	ì						
30 35	SEQ gtg	ID 1	NOS:	592 ( ggc	x 69. gag		ctg	ttc	acc	ggg	gtg	gtg					48	8
	SEQ gtg Val 1	agc Ser	aag Lys gac	ggc Gly ggc	gag Glu 5 gac	3 gag	ctg Leu aac	ttc Phe	acc Thr	ggg Gly 10	gtg Val ttc	gtg Val agc	Pro gtg	Ile tcc	Leu 15 ggc	Val gag	48	
35 40	SEQ gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr cac His 25	ggg Gly 10 aag Lys	gtg Val ttc Phe	gtg Val agc Ser	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	yal gag Glu tgc		5
35	gtg Val 1 gag Glu ggc Gly	agc Ser ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	ctg Leu aac Asn tac Tyr	ttc Phe ggc Gly ggc Gly 40	acc Thr cac His 25 aag Lys	ggg Gly 10 aag Lys ctg Leu	gtg Val ttc Phe acc Thr	gtg Val agc Ser ctg Leu	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96	5 4
35 40	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser ctg Leu gag Glu acc Thr 50 tac	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr gtg Val 55	ttc Phe ggc Gly ggc Gly 40 ccc Pro	acc Thr cac His 25 aag Lys tgg Trp	ggg Gly 10 aag Lys ctg Leu ccc Pro	gtg Val ttc Phe acc Thr	gtg Val agc Ser ctg Leu ctc Leu 60	gtg Val aag Lys 45 gtg Val	tcc ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	gag Glu tgc Cys	194 192 240	5 4

						gac Asp											336
5						acc Thr											384
10	gac Asp	ttc Phe 130	aag Lys	gag Glu	gac Asp	ggc Gly	aac Asn 135	atc Ile	ctg Leu	999 Gly	cac His	aag Lys 140	ctg Leu	gag Glu	tac Tyr	aac Asn	432
15						gtc Val 150	Tyr									•	474
20		F1D				372 <b>A</b> 5	muta	ation	ns								
25						gag Glu											48
						gac Asp											96
30						gcc Ala											144
35						ctg Leu											192
40						cag Gln 70											240
45						aag Lys											288
						aag Lys											336
50						gac Asp											384
55						gac Asp											432

							_			_	_	_	_	cag Gln			477
5					56₩,8 & 69	572 <b>A</b> 7	muta	ation	ns, M	Met (	) pos	s. 1	remo	oved			
10														atc Ile			48
15														tcc Ser 30			96
20														ttc Phe			144
20														acc Thr			192
25														atg Met			240
30														cag Gln			288
35					_	_	_				_		_	gcc Ala 110	 		336
40														aag Lys			384
40														gag Glu			432
45						gtc Val 150											474
50					ıtat: & .699												
55	atg	gtg	agc	aag	ggc	gag								ccc Pro		·	48

				gac Asp										96
5				gcc Ala										144
10				ctg Leu										192
15				cag Gln 70										240
20				aag Lys										288
	_			aag Lys	_	_				_	-	-		336
25				gac Asp										384
30				gac Asp										432
35				aac Asn 150										477
40		F1D,		ion, l	Met	@ pc	os. I	l rem	noved	Ē				
45				gag Glu										48
				gta Val										96
50				acc Thr										144
55				ccc Pro										192

				_	_	tgc Cys 70		_				_		_	_		:	240
5						tcc Ser											:	288
10					_	gac Asp	_						_	_				336
15						acc Thr												384
20						ggc Gly											4	432
						gtc Val 150											4	474
25		,																
		F1D,				nutat	ions	3										
	OEQ	ID I	105.	102 (	x / 0.	,												
30	atg	gtg	agc	aag	ggc	gag Glu												48
30 35	atg Met 1	gtg Val gag	agc Ser ctg	aag Lys gac	ggc Gly 5 ggc	gag	Glu gta	Leu	Phe ggc	Thr 10 cac	Gly	Val ttc	Val	Pro gtg	Ile 15 tcc	Leu		48 96
	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly	gag Glu gac	Glu gta Val acc	Leu aac Asn	Phe ggc Gly 25 ggc	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val 30	Ile 15 tcc Ser	ggc Gly	:	
35 40	atg Met 1 gtc Val gag Glu	gtg Val gag Glu ggc Gly	agc Ser ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp	gta Val acc Thr	aac Asn tac Tyr 40	ggc Gly 25 ggc Gly	Thr 10 cac His aag Lys	Gly aag Lys ctg Leu ccc	Val ttc Phe acc Thr	val agc ser ctg Leu 45 ctc	gtg Val 30 aag Lys	Ile 15 tcc ser ttc Phe	ggc Gly atc Ile acc		96
35 40 45	atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc ser ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly ggc	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala	gta Val acc Thr ccc Pro 55	Leu aac Asn tac Tyr 40 gtg Val	Phe ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr ccc	val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser ttc Phe acc Thr	ggc Gly atc Ile acc Thr	<u>:</u>	96 144
35 40 45	atg Met 1 gtc Val gag Glu tgc Cys ctc Leu 65	gtg Val gag Glu ggc Gly acc Thr 50 ggc Gly	agc Ser ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys ctg Leu	gag Glu gac Asp gcc Ala ctg Leu cag Gln	gta Val acc Thr ccc Pro 55 tgc Cys	Leu aac Asn tac Tyr 40 gtg Val ttc Phe	Phe ggc Gly 25 ggc Gly ccc Pro gcc Ala atg	Thr 10 cac His aag Lys tgg Trp cgc Arg	Gly aag Lys ctg Leu ccc Pro tac Tyr 75 gaa	Val ttc Phe acc Thr acc Thr 60 ccc Pro	val agc ser ctg Leu 45 ctc Leu gac Asp	gtg Val 30 aag Lys gtg Val cac	Ile 15 tcc Ser ttc Phe acc Thr atg Met	ggc Gly atc Ile acc Thr aag Lys 80 gag	:	96 144 192

						gac Asp											384
5		_		_		gac Asp				_			_	_			432
10						aac Asn 150											477
15	BFP	F1D,	, F64	4L,Y	56H 1	nutat	cions	3, Me	et @	pos	. 1 1	cemov	ved			•	
	SEQ	ID 1	NOS:	704 8	ž 70	5						•					
20						gag Glu											48
05						gta Val											96
25						acc Thr											144
30						ccc Pro											192
35						tgc Cys 70											240
40						tcc Ser											288
45						gac Asp											336
45						acc Thr											384
50	_		_		_	ggc Gly			-			_	_				432
55						gtc Val 150											474

BFP F1D, F64L, Y66H, Y145F mutations SEQ ID NOS:706 & 707

5				aag Lys													48
10				gac Asp 20													96
15				ggc Gly													144
20				ggc Gly													192
				ggc Gly												aag Lys 80	240
25				ttc Phe				_	-		_			_	_		288
30				ttc Phe 100													336
35	gtg Val	aag Lys	ttc Phe 115	gag Glu	ggc Gly	gac Asp	acc Thr	ctg Leu 120	gtg Val	aac Asn	cgc Arg	atc Ile	gag Glu 125	ctg Leu	aag Lys	ggc Gly	384
40				aag Lys													432
				agc Ser													477
45																	
	BFP SEQ			L, Y6			mut	atio	ons,	Met	@ pc	os. 1	. rem	nove	1		
50				ggc Gly												gtc Val	48
55				ggc Gly 20													96

				_	_	acc Thr			-	_	_	_		_	144	1
5				_	_	ccc Pro								ctc Leu	192	2
10						tgc Cys 70									240	)
15		_			_	tcc Ser	_	_		_		_	_	 _	288	3
20						gac Asp									336	5
						acc Thr									384	1
25						ggc Gly									432	3
30						gtc Val 150									474	1
35				15F r 710 8												
40	_		_	_		gag Glu		_			 			_	4 8	3
45						gac Asp									96	5
						gcc Ala									144	1
50						ctg Leu									192	2
55						cag Gln 70									240	)

				aag Lys											288
5				aag Lys											336
10				gac Asp											384
15				gac Asp											432
20				aac Asn 150											477
25		, Y14 NOS:		tion,	, Met	: @ r	pos.	1 re	emove	ed					
30				gag Glu											48
				gta Val											96
35				acc Thr			_	_		_	-			_	144
40				ccc Pro											192
45				tgc Cys 70											240
50	cac His			tcc Ser											288
50				gac Asp										gtg Val	336
55		 	 	200	cta	ata	220	000	ata	~~~	ata	226	ggc	ata	384

5			ggc Gly								432
Ū			gtc Val 150								474
10											
15		6L,F0	Y1451	₹ mut	atio	ons					
.0			gag Glu								48
20			gac Asp								96
25			gcc Ala								144
30			ctg Leu								192
35			cag Gln 70								240
55			aag Lys								288
40			aag Lys							gag Glu	336
45			gac Asp								384
50			gac Asp								432
55			aac Asn 150								477

BFP F1D, F46L,F64L,Y145F mutations, Met @ pos. 1 removed SEQ ID NOS:716 & 717

5														ctg Leu 15		48
10														ggc Gly		96
15														atc Ile		144
														acc Thr		192
20														aag Lys		240
25														gag Glu 95		288
30														gag Glu		336
35														ggc Gly		384
														tac Tyr		432
40			_			gtc Val 150		_	_	_	_	_	_			474
45	(YFE	P F2I	CO	resp	onds	@ po s to		dues	159-	-239	of Y	(FP)				
50	atg Met	aac	ggc		aag Lys	gtg			Ile					gag Glu		48
55														atc Ile		96

		ggc Gly											144
5		ctg Leu 50										ctg Leu	192
10		ttc Phe											240
15	aag Lys											٠	243
		F2D											
20		ggc Gly											48
25		gtg Val											96
30		ccc Pro											144
35		agc Ser 50											192
00		gtg Val											240
40		F2D,			Met	ado	ded (	pos	s. 1				
45		aac Asn											48
~_1		agc Ser	_	_				_	_				96
50		ggc Gly											144
55		ctg Leu 50											192

5					_	gcc Ala 70						_	_		_		240
	aag Lys																243
10				03H r 724 8					÷								
15				_		aac Asn		_		_					_		48
20						gac Asp											96
25				_	_	ccc Pro	_				_	_		_		_	144
	_	_		_		aac Asn	_	_	_	_		_	_	_	_		192
30						999 Gly 70											240
35				03H r 726 8		ion,	. Met	ado	ded (	oq @	s. 1						
40						gtg Val											48
45						gcc Ala											96
						ctg Leu	Pro										144
50	_	_	_		_	ccc Pro			-	_	_		_	_	_	_	192
55						gcc Ala 70											240

	aag Lys																243
5		F2D ID 1															
10						aac Asn											48
15						gac Asp											96
20						ccc Pro											144
20						aac Asn										gag Glu	192
25						ggg Gly 70											240
30		F2D ID 1				ion,	Met	ado	ded (	) pos	s. 1						
35		aac															
	1	Asn				gtg Val											48
40	1 ggc	agc	Gly gtg	Ile cag	Lys 5 ctc		Asn gac	Phe cac	Lys	Ile 10 cag	Arg	His aac	Asn	Ile	Glu 15 atc	Asp	48 96
40	ggc Gly	agc Ser ggc	Gly gtg Val ccc	cag Gln 20	Lys 5 ctc Leu ctg	Val gcc	Asn gac Asp	Phe cac His	tac Tyr 25	Ile 10 cag Gln cac	Arg cag Gln tac	His aac Asn	Asn acc Thr	CCC Pro 30	Glu 15 atc Ile	Asp ggc Gly tcc	
40 45	ggc Gly gac Asp	agc Ser ggc Gly	gtg Val ccc Pro 35	cag Gln 20 gtg Val	Lys 5 ctc Leu ctg Leu	Val gcc Ala ctg	Asn gac Asp ccc Pro	Phe cac His gac Asp 40	tac Tyr 25 aac Asn	Ile 10 cag Gln cac His	arg cag Gln tac Tyr gat	aac Asn ctg Leu	acc Thr agc ser 45	CCC Pro 30 acc Thr	Glu 15 atc Ile cag Gln	Asp ggc Gly tcc Ser	96
	ggc Gly gac Asp gcc Ala	agc Ser ggc Gly ctg Leu 50	gtg Val ccc Pro 35 agc Ser	cag Gln 20 gtg Val aaa Lys	Lys 5 ctc Leu ctg Leu gac Asp	yal gcc Ala ctg Leu	Asn gac Asp ccc Pro aac Asn 55	Phe cac His gac Asp 40 gag Glu	tac Tyr 25 aac Asn aag Lys	Ile 10 cag Gln cac His cgc Arg	arg cag Gln tac Tyr gat Asp	His aac Asn ctg Leu cac His 60 atg	Asn acc Thr agc ser 45 atg Met	CCC Pro 30 acc Thr gtc Val	Glu 15 atc Ile cag Gln ctg Leu	Asp ggc Gly tcc Ser ctg Leu tac	96 144

## CFP F2D, V163A,Y203T mutations SEQ ID NOS:732 & 733

5				_	_	aac Asn		_		_					_		48
10						gac Asp											96
15				_	_	ccc Pro	_				_	_		_		_	144
.0						aac Asn											192
20						999 Gly 70											240
25	CFP	ESD	<b>V</b> 14	532 1	72035	Г, М∈	at ad	na h	@ n	ne 1	ı					·	
				734 8			st at	ueu	⊕ Þ¢	JS	_						
30						gcc Ala					-					_	48
35	Met 1 ggc	Asn agc	Gly gtg	Ile cag	Lys 5 ctc		Asn gac	Phe cac	Lys tac	Ile 10 cag	Arg cag	His aac	Asn	Ile	Glu 15 atc	Asp	48 96
	Met 1 ggc Gly gac	Asn agc Ser	gtg Val	cag Gln 20	Lys 5 ctc Leu ctg	Ala	Asn gac Asp	Phe cac His	tac Tyr 25	Ile 10 cag Gln cac	arg cag Gln tac	His aac Asn	Asn acc Thr	CCC Pro 30	Glu 15 atc Ile cag	Asp ggc Gly tcc	
35 40	Met 1 ggc Gly gac Asp	agc ser ggc Gly	gtg Val ccc Pro 35	cag Gln 20 gtg Val	Lys 5 ctc Leu ctg Leu	Ala gcc Ala ctg	Asn gac Asp ccc Pro	Phe cac His gac Asp 40	tac Tyr 25 aac Asn	Ile 10 cag Gln cac His	arg cag Gln tac Tyr	aac Asn ctg Leu	acc Thr agc ser 45	CCC Pro 30 acc Thr	Glu 15 atc Ile cag Gln	Asp ggc Gly tcc Ser	96
35	Met 1 ggc Gly gac Asp gcc Ala	agc ser ggc Gly ctg Leu 50	gtg Val ccc Pro 35 agc ser	cag Gln 20 gtg Val aaa Lys	Lys 5 ctc Leu ctg Leu gac Asp	Ala gcc Ala ctg Leu	Asn gac Asp ccc Pro aac Asn 55	Phe cac His gac Asp 40 gag Glu atc	tac Tyr 25 aac Asn aag Lys	Ile 10 cag Gln cac His cgc Arg	arg cag Gln tac Tyr gat Asp	His aac Asn ctg Leu cac His 60 atg	Asn acc Thr agc ser 45 atg Met	CCC Pro 30 acc Thr gtc Val	Glu 15 atc Ile cag Gln ctg Leu	Asp ggc Gly tcc Ser ctg Leu tac	96 144

Venus F2D, S175G mutation SEQ ID NOS:736 & 737

5				aac Asn											48
10				gac Asp											96
15				ccc Pro											144
				aac Asn											192
20				999 Gly 70					_	_		_		_	240
25		ıs F2 ID 1		tatio	on, N	Met a	addeo	d @ p	os.	1	. •				
30				gtg Val											48
35				gcc Ala											96
				ctg Leu											144
40				ccc Pro			_	_	_		_	_	_	_	192
45				gcc Ala 70											240
50	aag Lys					-									243
		ıs F2 ID N		75G n L	nutat	ions	5								
55				aac Asn											48

5				ctc Leu 20												96
				ctg Leu												144
10				gac Asp												192
15				gcc Ala												240
20				/163/ /42 8			Met	adde	ed @	pos	. 1					
25				atc Ile		-			_		_				_	48
20				cag Gln 20						_	_					96
30				gtg Val												144
35				aaa Lys												192
40				acc Thr												240
	aag Lys															243
45	•															
50	(YFI	P F1E	Cor	os. 1 rres <u>r</u> 744 8	onds	s to			lues	1-17	74 of	YFI	?)			
30				ggc Gly												48
55	gag Glu			ggc Gly 20												96

5										atc Ile		144
										acc Thr		192
10										aag Lys		240
15										gag Glu 95		288
20										gag Glu		336
25										ggc Gly		384
										tac Tyr		432
30										aac Asn		480
35				aag Lys								519
40		9R mi 746 8										
45										atc Ile 15		.48
40										tcc Ser		96
50	 	 	_	_		 _	_	_	_	ttc Phe		144
55				Leu						acc Thr		192

						cag Gln 70												240
5						aag Lys												288
10						aag Lys												336
15						gac Asp												384
20						gac Asp												432
						aac Asn 150											-	480
25						ttc Phe									•			522
30				9R mi 748 8		ion,	pos	. 1 1	Met 1	cemov	red							
30 35	SEQ gtg	ID Nagc	NOS:	748 8 ggc	x 749		ctg	ttc	acc	999	gtg							48
	gtg Val 1 gag	agc Ser	aag Lys gac	ggc Gly ggc	gag Glu 5 gac	gag	ctg Leu aac	ttc Phe ggc	acc Thr	999 Gly 10 aag	gtg Val ttc	Val agc	Pro gtg	Ile tcc	Leu 15 ggc	Val gag		48 96
35	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20 gat	gag Glu 5 gac Asp	gag Glu gta	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr cac His 25	ggg Gly 10 aag Lys	gtg Val ttc Phe	Val agc Ser	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc		
35	gtg Val 1 gag Glu ggc Gly	agc Ser ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	ctg Leu aac Asn tac Tyr	ttc Phe ggc Gly ggc Gly 40 ccc	acc Thr cac His 25 aag Lys	ggg Gly 10 aag Lys ctg Leu	gtg Val ttc Phe acc Thr	val agc ser ctg Leu ctc	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys		96
35	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser ctg Leu gag Glu acc Thr 50 tac	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr gtg Val 55	ttc Phe ggc Gly ggc Gly 40 ccc Pro	acc Thr cac His 25 aag Lys tgg Trp	999 Gly 10 aag Lys ctg Leu ccc Pro	gtg Val ttc Phe acc Thr	val agc ser ctg Leu ctc Leu 60 gac	gtg Val aag Lys 45 gtg Val	tcc ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ttc Phe		96 144

						gac Asp												336
5						acc Thr											•	384
10						ggc Gly											•	432
15	tac Tyr 145	aac Asn	agc Ser	cac His	aac Asn	gtc Val 150	tat Tyr	atc Ile	atg Met	gcc Ala	gac Asp 155	aag Lys	cag Gln	aag Lys	aac Asn	ggc Gly 160	4	480
20						aag Lys									·		!	519
	YFP	F1E	, Y60	6F mi	ıtat:	ion												
25	SEQ	ID 1	NOS:	750 8	£ 75:	1												
						gag Glu												48
30						gac Asp												96
35						gcc Ala											=	144
40					_	ctg Leu		,									-	192
45	ttc Phe 65	ggc Gly	ttc Phe	ggc Gly	ctg Leu	cag Gln 70	tgc Cys	ttc Phe	gcc Ala	cgc Arg	tac Tyr 75	ccc Pro	gac Asp	cac His	atg Met	aag Lys 80	2	240
						aag Lys											2	288
50						aag Lys											3	336·
55						gac Asp											. 3	384

					gac Asp											432
5			_		aac Asn 150	_			_	_		_	_	_	aac Asn 160	480
10					ttc Phe											522
15			5F mi 752 {		ion,	pos	. 1 1	Met 1	remo	ved						·
20					gag Glu											48
05					gta Val											96
25		 	_	_	acc Thr			_	-		_	_			_	144
30					ccc Pro											192
35					tgc Cys 70											240
40				_	tcc Ser	_	_		_			_	_		_	288
<b>A</b> E					gac Asp											336
45					acc Thr											384
50	_	_		_	ggc Gly			_			_	_				432
55					gtc Val 150											480

				aag Lys									519
5						٠							
			9K mi 754 {										
10	_	_	_	 		_		 		atc Ile 15	_		48
15										tcc Ser			96
20										ttc Phe		1	144
25										acc Thr	acc Thr	1	L <b>92</b>
										atg Met		2	240
30										cag Gln 95		2	88
35				_	_	_		_	_	gcc Ala		3	336
40										aag Lys		3	884
45										gag Glu		4	32
										aag Lys		4	80
50				ttc Phe								5	522

YFP F1E, Q69K mutation, pos. 1 Met removed SEQ ID NOS:756 & 757

5				gag Glu									4	8
10				gta Val									91	6
15	 	 _	_	acc Thr			_		_	_		_	144	4
				ccc Pro									19	2
20				tgc Cys 70	Phe								240	0
25				tcc Ser									288	8
30			_	gac Asp	_			_		_	-	 	336	6
35				acc Thr									384	4
00				ggc Gly									432	2
40				gtc Val 150									480	0
45				aag Lys									519	9
50			3L,Q0	59M r	nutat	ions	3							
55				gag Glu									4.8	8

				gac Asp											·	96
5				gcc Ala												144
10				ctg Leu												192
15				atg Met 70				-			_		_	_		240
20				aag Lys												288
				aag Lys												336
25				gac Asp												384
30				gac Asp												432
35			_	aac Asn 150	_				_	_	_	_	_			480
40	_	_		ttc Phe	_		_					_				522
45			E,V68	59M n	nutat	cions	s, po	os. 3	L Met	ren	noved	i				
				gag Glu												48
50				gta Val												96
55				acc Thr										tgc Cys		144

														acc Thr		192
5														atg Met		240
10														cag Gln		288
15					_	_	_				_		_	gcc Ala 110	 	336
20									Asn					aag Lys		384
														gag Glu		432
25			_			_			-	-	_	_	_	aag Lys		480
30		_				aag Lys		_					_		·	519
35				1L mι 762 δ												
40														ccc Pro		48
45	_		-	_		_	_				_		_	gtg Val 30		96
														aag Lys		144
50														gtg Val		192
55														cac His		240

			_			aag Lys		-	_		_		_	_		288
5						aag Lys										336
10						gac Asp										384
15		_		_		gac Asp				_		_	_			432
20						aac Asn 150										480
20	Gly	Ile	Lys	Val	Asn 165	ttc Phe	Lys	Ile	Arg	His 170	Asn		_			522
25			, F64 NOS:			ion,	pos.	. 1 M	Met 1	cemov	rea					
30						gag Glu										48
00						gta Val										96
35						acc Thr										144
40				_	_	ccc Pro									_	192
45						tgc Cys 70										240
	cac His					tcc Ser										288
50						gac Asp										336
55						acc Thr										384

5			ggc Gly							•	432
			gtc Val 150								480
10			aag Lys								519
15		54L,8 766 8	, Y66V 7	√ mut	atio	ons					
20			gag Glu							,	48
25			gac Asp								96
20			gcc Ala								144
30			ctg Leu								192
35			cag Gln 70								240
40			aag Lys								288
45			aag Lys								336
-10			gac Asp								384
50			gac Asp								432
55			aac Asn 150							,	480

5			gtg Val												522
	CFP SEQ		64L,8 768			W mut	tatio	ons,	pos	. 1 1	Met 1	remo	ved		
10			ggc Gly												48
15			ggc Gly 20												96
20			gat Asp												144
25			aag Lys											ctg Leu	192
_			ctg Leu												240
30			ttc Phe												288
35			ttc Phe 100	_	_	_				_		_	_	 	336
40			ggc Gly												384
45			gag Glu												432
			cac His												480
50			aac Asn												519

CFP F1E, F64L,S65T,Y66W,N146I,M153T, V163A mutations SEQ ID NOS:770 & 771

5									ttc Phe								48
10									ggc Gly 25								96
15									ggc Gly								144
	tgc .Cys								ccc Pro								192
20									gcc Ala								240
25									atg Met								288
30	_					_	_	_	ggc Gly 105			_		_	_		336
35									gtg Val								384
									atc Ile								432
40									atc Ile								480
45									cgc Arg								522
50	CFP	F1E,	, F64	1L,S	55T,	166W,	. N146	5I,M1	L53T,	.V163	3A mi	ıtat:	ions	, pos	s. 1	Met	removed
	SEQ	ID 1	10S:	772 8	k 773	3											
55									acc Thr								48

	 _	_		_	gta Val				_		_			 		96
5	 		_	-	acc Thr			_	_		_	_		_	1	44
10					ccc Pro										1	92
15					tgc Cys 70										2	40
20					tcc Ser										2	88
_				_	gac Asp	_				_		_	_	 	3	36
25					acc Thr										3	84
30					ggc Gly										4	32
35					gtc Val 150										4	80
40					aag Lys		_					_			5	19
45			5₩ mi 774 8													
					gag Glu											48
50					gac Asp										,	96
55					gcc Ala									atc Ile	1	44

			ctg Leu									192
5			cag Gln 70									240
10			aag Lys									288
15			aag Lys									336
20			gac Asp			Val						384
20	_	_	 gac Asp				_		_	_		432
25			aac Asn 150									480
30			ttc Phe									522
35		√ mut 776 &	on, g 7	pos.	1 Me	et re	emove	ed				
40			gag Glu									48
45			gta Val									96
			acc Thr									144
50			ccc Pro									192
55			tgc Cys 70									240

						tcc Ser											288
5						gac Asp											336
10						acc Thr											384
15						ggc Gly											432
20						gtc Val 150											480
						aag Lys											519
25																	
		F1E,				I mut	atio	ons									
30					ggc	gag Glu											48
30 35	Met 1 gtc	Val gag	Ser	Lys gac	ggc Gly 5 ggc	gag	Glu gta	Leu	Phe ggc	Thr 10 cac	Gly aag	Val ttc	Val agc	Pro gtg	Ile 15 tcc	Leu	<b>48</b> 96
	Met 1 gtc Val gag	Val gag Glu ggc	ser ctg Leu	Lys gac Asp 20	ggc Gly 5 ggc Gly	gag Glu gac	Glu gta Val acc	Leu aac Asn	Phe ggc Gly 25	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val 30	Ile 15 tcc ser	Leu ggc Gly atc	
35 40	Met 1 gtc Val gag Glu	yal gag Glu ggc Gly	Ser ctg Leu gag Glu 35	gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp	Glu gta Val acc Thr	Leu aac Asn tac Tyr 40	Phe ggc Gly 25 ggc Gly ccc	Thr 10 cac His aag Lys	aag Lys ctg Leu	ttc Phe acc Thr	agc ser ctg Leu 45	gtg Val 30 aag Lys	tcc ser ttc Phe	Leu ggc Gly atc Ile	96
35 40 45	Met 1 gtc Val gag Glu tgc Cys	yal gag Glu ggc Gly acc Thr 50	ser ctg Leu gag Glu 35 acc Thr	Lys gac Asp 20 ggc Gly ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala	gta Val acc Thr ccc Pro 55	Leu aac Asn tac Tyr 40 gtg Val	Phe ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	agc ser ctg Leu 45 ctc Leu	gtg Val 30 aag Lys gtg Val	Ile 15 tcc ser ttc Phe acc Thr	ggc Gly atc Ile acc Thr	96 144
35 40 45	Met 1 gtc Val gag Glu tgc Cys ttc Phe 65 cgg	yal gag Glu ggc Gly acc Thr 50 ggc Gly	ser ctg Leu gag Glu 35 acc Thr tgg Trp	Lys gac Asp 20 ggc Gly ggc Gly ttc	ggc Gly 5 ggc Gly gat Asp aag Lys ctg Leu	gag Glu gac Asp gcc Ala ctg Leu cag Gln	Glu gta Val acc Thr ccc Pro 55 tgc Cys	Leu aac Asn tac Tyr 40 gtg Val ttc Phe	Phe ggc Gly 25 ggc Gly ccc Pro gcc Ala atg	Thr 10 cac His aag Lys tgg Trp cgc Arg	Gly aag Lys ctg Leu ccc Pro tac Tyr 75 gaa	Val ttc Phe acc Thr acc Thr 60 ccc Pro	agc Ser ctg Leu 45 ctc Leu gac Asp	gtg Val 30 aag Lys gtg Val cac	Ile 15 tcc Ser ttc Phe acc Thr atg Met	ggc Gly atc Ile acc Thr aag Lys 80 gag	96 144 192

				acc Thr										384
5				ggc Gly 135										432
10				gtc Val										480
15		_		aag Lys		_					-			522
20			N146: & 78:	tatio	ons,	pos	. 1 1	Met 1	remov	ved				
25				ctg Leu										48
				aac Asn										96
30 -				tac Tyr										144
35				gtg Val 55										192
40				ttc Phe									:	240
45				gcc Ala									:	288
				gac Asp										336
50				ctg Leu										384
55				aac Asn 135										432

			_			gtc Val 150		_	_	_	 _	_			•	480
5		_				aag Lys					 _					519
10				53T t 782 ≀												
15						gag Glu										48
20	_		_	_		gac Asp	_			_	_					96
20					_	gcc Ala			_	_	_	_				144
25	_				_	ctg Leu										192
30					_	cag Gln 70	_	_	_		_		_	_		240
35						aag Lys										288
40						aag Lys										336
10						gac Asp										384
45						gac Asp										432
50						aac Asn 150										480
55						ttc Phe										522

CFP F1E, M153T mutation, pos. 1 Met removed SEQ ID NOS:784 & 785

5				gag Glu									48
10				gta Val									96
15				acc Thr									144
				ccc Pro									192
20				tgc Cys 70									240
25	_		_	tcc Ser	_	_	_			_	_	 -	288
30			_	gac Asp	-								336
35				acc Thr									384
00				ggc Gly									432
40		_		gtc Val 150			_	_	_				480
45				aag Lys									519
50	F1E ID I			3 <b>T m</b> ւ 7	ıtat:	ions							
				gag Glu									48
55				gac Asp									96

5	ggc													144
	acc Thr 50		_	_										192
10	ggc													240
15	cac His													288
20	acc Thr													336
25	aag Lys												ggc Gly	384
20	gac Asp 130													432
30	tac Tyr													480
35	atc Ile	_			_		_					_		522
40	F1E,				ıtati	ons,	pos	3. 1	Met	remo	oved			
45	agc Ser													48
	ctg Leu													96
50	gag Glu													144
55	acc Thr 50													192

						tgc Cys 70											240
5						tcc Ser											288
10					_	gac Asp	_				_		_	_			336
15						acc Thr											384
20						ggc Gly											432
						gtc Val 150											480
25						aag Lys											519
30 -			E, NI NOS:			53T, L	V163	BA mi	utati	ions							
30	SEQ atg	ID 1	10S:	790 8 aag	x 79:		gag	ctg	ttc	acc							48
	sEQ atg Met 1.	ID i gtg Val gag	agc Ser ctg	aag Lys gac	ggc Gly 5 ggc Gly	l gag	gag Glu gta Val	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr 10 cac	Gly aag Lys	Val ttc Phe	Val agc Ser	Pro gtg	Ile 15 tcc	Leu ggc	48 96
35 40	atg Met 1. gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly gat	gag Glu gac Asp	gag Glu gta Val	ctg Leu aac Asn	ttc Phe ggc Gly 25	acc Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val 30 aag	Ile 15 tcc Ser	ggc Gly atc	
35	sEQ atg Met 1. gtc Val gag Glu tgc	gtg Val gag Glu ggc Gly acc	agc Ser Ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr 40	ttc Phe ggc Gly 25 ggc Gly	acc Thr 10 cac His aag Lys	Gly aag Lys ctg Leu ccc	Val ttc Phe acc Thr	val agc ser ctg Leu 45	gtg Val 30 aag Lys	tcc ser ttc Phe	ggc Gly atc Ile	96
35 40	sEQ atg Met 1. gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser Ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala	gag Glu gta Val acc Thr ccc Pro 55	ctg Leu aac Asn tac Tyr 40 gtg Val	ttc Phe ggc Gly 25 ggc Gly ccc Pro	acc Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	Val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc ser ttc Phe acc Thr	ggc Gly atc Ile acc Thr	96 144

					aag Lys											336
5	 _				gac Asp		-			_			_	_		384
10					gac Asp											432
15					aac Asn 150											480
20		_	_		ttc Phe	_		_					_			522
25			16I, 792 8		3T, V 3	/163/	A mut	atio	ons,	pos	. 1 N	Met 1	cemov	red		
					gag Glu											48
30					gta Val											96
35					acc Thr											144
40					ccc Pro											192
45			_	_	tgc Cys 70		_	_			_		_			240
.0	_			_	tcc Ser	_	_		_			_	_		_	288
50					gac Asp											336
55					acc Thr											384

			ggc Gly									432
5			gtc Val 150									480
10			aag Lys									519
15	F1E,		,M153	3T mi	ıtat:	ions						
20			gag Glu									48
05			gac Asp									96
25			gcc Ala									144
30			ctg Leu									192
35			cag Gln 70									240
40			aag Lys									288
45			aag Lys	_	-			_		_	_	 336
43			gac Asp									384
50			gac Asp									432
55		_	aac Asn 150	_			_	_	_	_	_	480

			_				_		-	cac His 170				_			5	22
5																		
				6W, 1 796 8			nd I	11537	r mut	tatio	ons,	pos	. 1 1	Met 1	cemo	ved		
10										ggg Gly 10								48
15										aag Lys								96
20										ctg Leu							1	44
25										ccc Pro							1	92
										tac Tyr							2	40
30										gaa Glu 90							2	88
35					_	_	-			tac Tyr	_		_	_			3	36
40										cgc Arg							3	84
45										Gly 999							4.	32
	tac Tyr 145	atc Ile	agc Ser	cac His	aac Asn	gtc Val 150	tat Tyr	atc Ile	acc Thr	gcc Ala	gac Asp 155	aag Lys	cag Gln	aag Lys	aac Asn	ggc Gly 160	4	80
50										aac Asn 170							5	19

CFP F1E, Y66W, N146I, M153T, V163A mutations SEQ ID NOS:798 & 799

5													ccc Pro			48
10	_	 _	_		_	_				_		_	gtg Val 30			96
15		 		_	_				_	_		_	aag Lys			144
													gtg Val			192
20													cac His			240
25													gtc Val			288
30					_	_	_				_		cgc Arg 110	_		336
35													ctg Leu			384
													ctg Leu			432
40													cag Gln			480
45		_	_	aac Asn 165		_		_					_			522
50				N1461 & 801		.53T,	. V16	53Α π	nutat	ions	s, po	os. I	L Met	: rem	noved	
55													atc Ile			48

			gta Val							,	96
5			acc Thr								144
10			ccc Pro								192
15			tgc Cys 70								240
20			tcc Ser								288
20			gac Asp						gtg Val		336
25			acc Thr								384
30			ggc Gly								432
35			gtc Val 150								480
40			aag Lys								519
ΛE		5 <b>Α</b> πι 302 δ		•							
45			gag Glu								48
50			gac Asp								96 <sup>.</sup>
55			gcc Ala						atc Ile		144

				ctg Leu									192
5	_		_	cag Gln 70	_		-	_		_	_	_	240
10				aag Lys									288
15				aag Lys									336
20				gac Asp									384
				gac Asp									432
25				aac Asn 150									480
30				ttc Phe									522
35		5A mi 304 8		ion,	pos.	. 1 N	Met 1	cemov	ved				
40				gag Glu									48
45				gta Val									96
				acc Thr									144
50				ccc Pro								ttc Phe	192
55				tgc Cys 70								cgg Arg 80	240

				ttc Phe													288
5				ttc Phe 100													336
10	_			ggc Gly	_		-			_			_	_			384
15				gag Glu													432
20				cac His													480
20		_		aac Asn		_		_					_				519
25																	
		F1E,		5 <b>A</b> , 3			2A mu	ıtat	ions								
	~ <b>_</b> x					,											
30	atg	gtg	agc	aag Lys	ggc	gag											48
35	atg Met 1 gtc	gtg Val gag	agc Ser ctg	aag	ggc Gly 5	gag Glu gac	Glu gta	Leu	Phe ggc	Thr 10 cac	Gly aag	Val ttc	Val agc	Pro gtg	Ile 15 tcc	Leu ggc	48 96
	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly 5 ggc Gly gat	gag Glu gac Asp	Glu gta Val acc	Leu aac Asn	ggc Gly 25	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	val agc ser	Pro gtg Val 30	Ile 15 tcc Ser	Leu ggc Gly atc	
35 40	atg Met 1 gtc Val gag Glu	gtg Val gag Glu ggc Gly acc	agc Ser Ctg Leu gag Glu 35	aag Lys gac Asp 20	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp gcc Ala	Glu gta Val acc Thr	aac Asn tac Tyr 40	ggc Gly 25 ggc Gly	Thr 10 cac His aag Lys	aag Lys ctg Leu	ttc Phe acc Thr	agc Ser ctg Leu 45	gtg Val 30 aag Lys	tcc ser ttc Phe	ggc Gly atc Ile	96
35	atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser Ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala ctg Leu	gta Val acc Thr ccc Pro 55	Leu aac Asn tac Tyr 40 gtg Val	ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr ccc	Val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser ttc Phe acc Thr	ggc Gly atc Ile acc Thr	96
35 40	atg Met 1 gtc Val gag Glu tgc Cys ttc Phe 65	gtg Val gag Glu ggc Gly acc Thr 50 gcc Ala	agc Ser Ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys ctg Leu	gag Glu gac Asp gcc Ala ctg Leu cag Gln 70	Glu gta Val acc Thr ccc Pro 55 tgc Cys	Leu aac Asn tac Tyr 40 gtg Val ttc Phe	ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp cgc Arg	Gly aag Lys ctg Leu ccc Pro tac Tyr 75 gaa	ttc Phe acc Thr acc Thr 60 ccc Pro	Val agc Ser ctg Leu 45 ctc Leu gac Asp	gtg Val 30 aag Lys gtg Val cac His	Ile 15 tcc Ser ttc Phe acc Thr atg Met	ggc Gly atc Ile acc Thr aag Lys 80 gag	96 144 192

													aag Lys		384
5													gag Glu		432
10													aag Lys		480
15			 		_	atc Ile	_					_			522
20				466₩, & 809	B 572	2A mi	ıtat:	ions	, pos	3. 1	Met	remo	oved		
25													ctg Leu 15	gtc Val	48
													ggc Gly		96
30													atc Ile		144
35	acc Thr												acc Thr		192
40													aag Lys		240
45		_		_	_	_		_			_	_	gag Glu 95	_	288
40													gag Glu		336
50	_		 	_	_	-		_			_	_	ggc Gly		384
55													tac Tyr		432

			aac Asn											4	480
5			ttc Phe 165												519
10			Y66W & 81:	2 <b>A</b> , 1	N1461	[, M]	153T	, and	d V16	53A r	mutai	cions	3		
15			ggc Gly 5												48
20			ggc Gly												96
25			gat Asp											1	144
20			aag Lys											1	192
30 -			ctg Leu											2	240
35			ttc Phe 85											2	288
40			ttc Phe											3	336
45			ggc Gly											3	384
70			gag Glu											4	132
50		_	cac His	_				_	_	_	_	_		4	180
55			aac Asn 165											5	522

5				5A,Y			, N146	6I,M:	153T	,V16:	3A mi	ıtat:	ions	, pos	. 1 1	Met r	remove	ed
	gtg Val 1	agc Ser	aag Lys	ggc Gly	gag Glu 5	gag Glu	ctg Leu	ttc Phe	acc Thr	999 Gly 10	gtg Val	gtg Val	ccc Pro	atc Ile	ctg Leu 15	gtc Val		48
10							aac Asn											96
15							tac Tyr											144
20							gtg Val 55											192
25							ttc Phe											240
							gcc Ala											288
30							gac Asp											336
35							ctg Leu											384
40							aac Asn 135											432
45							tat Tyr											4.80
							atc Ile											519
50				5Η mι 314 δ														
55							gag .Glu											48

				gac Asp 20											96
5				ggc Gly										atc Ile	144
10				ggc Gly											192
15				ggc Gly											240
20				ttc Phe											288
				ttc Phe 100											336
25				gag Glu											384
30				aag Lys											432
35				agc Ser											480
40				gtg Val											522
	BFP	F1E,	. Y66	5H mu	ıtat:	ion,	pos.	. 1 N	let 1	cemov	red				
45				316 8											
				ggc											48
50				ggc Gly 20											96
55				gat Asp											144

									acc Thr		192
5									atg Met		240
10									cag Gln		288
15									gcc Ala 110		336
20									aag Lys		384
									gag Glu	aac Asn	432
25									aag Lys		480
30			aag Lys								519
35		łL,Y6 318 8	nutat Ə	ions	5						
40		Lys	Glu		Leu	Thr	Gly		ccc Pro		48
45									gtg Val 30		96
									aag Lys		144
50									gtg Val	acc Thr	192
55									cac His	aag Lys 80	240

			_			aag Lys		_	_		_			_	_		288
5						aag Lys										gag Glu	336
10		_				gac Asp		-			_			_	_		384
15		_		_		gac Asp				_			_	_			432
20						aac Asn 150											480
20			_			ttc Phe	_		_					_			522
25															•		
		F1E				nutat 1	ions	s, po	os. :	1 Met	ren	nove	i				
	_				. 01.	-											
30	gtg	agc	aag	ggc	gag	gag Glu											48
30 35	gtg Val 1	agc Ser ctg	aag Lys gac	ggc Gly	gag Glu 5 gac	gag	Leu	Phe ggc	Thr	Gly 10 aag	Val ttc	Val agc	Pro gtg	Ile	Leu 15 ggc	Val gag	96
·	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	Leu aac Asn	Phe ggc Gly ggc	Thr cac His 25	Gly 10 aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
35 40	gtg Val 1 gag Glu ggc Gly	agc Ser Ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	aac Asn tac Tyr	ggc Gly ggc Gly 40	Thr cac His 25 aag Lys tgg	Gly 10 aag Lys ctg Leu	ttc Phe acc Thr	agc Ser ctg Leu	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96
35	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	Leu aac Asn tac Tyr gtg Val 55	ggc Gly ggc Gly 40 ccc Pro	Thr cac His 25 aag Lys tgg Trp cgc	Gly 10 aag Lys ctg Leu ccc Pro	ttc Phe acc Thr	Val agc Ser ctg Leu ctc Leu 60 gac	gtg Val aag Lys 45 gtg Val	tcc ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ctc Leu cgg	96 144
35 40	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50 cac His	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly 20 gat Asp aag Lys ctg Leu	gag Glu 5 gac Asp gcc Ala ctg Leu cag Gln	gag Glu gta Val acc Thr ccc Pro	Leu aac Asn tac Tyr gtg Val 55 ttc Phe gcc	ggc Gly 40 ccc Pro	Thr cac His 25 aag Lys tgg Trp cgc Arg	Gly 10 aag Lys ctg Leu ccc Pro tac Tyr	ttc Phe acc Thr acc Thr	Val agc Ser ctg Leu ctc Leu 60 gac Asp	gtg Val aag Lys 45 gtg Val cac	tcc Ser 30 ttc Phe acc Thr atg Met	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ctc Leu cgg Arg 80 cgc	96 144 192

	_	ttc Phe			_		_			_			_	_			384
5		ttc Phe 130															432
10		aac Asn	_			_			_	_	_	_	_	-			480
15		aag Lys															519
20		F1E					15F n	nutat	ions	5							
25	_	gtg Val	_	_				_								ctg Leu	48
20		gag Glu															96
30		ggc Gly															144
35		acc Thr 50															192
40		ggc Gly															240
45		cac His	_			_		_	_		_				_		288
10		acc Thr															336
50		aag Lys				_		_			_			_	_	ggc Gly	384
55		gac Asp 130				Asp											432

				aac Asn 150											480
5				ttc Phe											522
10			¥66H ⊊ 825	, Y14 5	15F r	nutat	cions	s, po	os. 3	l Met	rer	nove	i .		
15	 _	_	 	gag Glu	-								_	_	48
20				gta Val											96
				acc Thr											144
25				ccc Pro											192
30				tgc Cys 70											240
35				tcc Ser											288
40				gac Asp											336
				acc Thr	Leu										384
45				ggc Gly											432
50				gtc Val 150											480
55				aag Lys											519

## BFP F1E, Y145F mutation SEQ ID NOS:826 & 827

5														ccc Pro			48
10														gtg Val 30			96
15														aag Lys			144
	_				_	_								gtg Val			192
20														cac His			240
25			_			_		_	_		_			gtc Val	_		288
30												_		cgc Arg 110	_		336
35														ctg Leu			384
														ctg Leu			432
40				_			_			_	_	_	_	cag Gln	_		480
45					aac Asn 165											·	522
50	BFP	F1E.	. Y14	15F n	nutat	ion	200	s. 1	Met	remo	oved						
					829		<u> </u>										
55														atc Ile			48

		ggc Gly 20									96
5		gat Asp									144
10		aag Lys									192
15		ctg Leu									240
20		ttc Phe									288
		ttc Phe 100								gtg Val	336
25		ggc Gly									384
30		gag Glu									432
35		cac His									480
40	_	 aac Asn	_		-			_			519
45		F46L 330 8		1.							
		aag Lys									48
50		gac Asp 20									96
55		ggc Gly									144

		ggc Gly											192
5		ggc Gly											240
10		ttc Phe											288
15		ttc Phe 100											336
20		gag Glu											384
		aag Lys											432
25		agc Ser											480
30		gtg Val											522
35		F46L 332 8		ı, po	os. I	L Met	rer	nove	Ė				
40		ggc Gly											48
45		ggc Gly 20											96
<b>70</b>		gat Asp		Tyr			_		_	-	_	_	144
50		aag Lys											192
55		ctg Leu											240

						tcc Ser											288
5						gac Asp											336
10						acc Thr											384
15						ggc Gly											432
20						gtc Val 150											480
		_				aag Lys		_					_			÷	519
25															•		
		ıs Fi				L mut	atio	ons									
	SEQ	10 1	NOS: 6	334 (	x 03:	ر											
30	atg	gtg	agc	aag	ggc	gag Glu											48
30 -	atg Met 1 gtc	gtg Val gag	agc Ser	aag Lys gac	ggc Gly 5	gag	Glu gta	Leu aac	Phe ggc	Thr 10 cac	Gly aag	Val ttc	Val agc	Pro gtg	Ile 15 tcc	Leu ggc	48 96
	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly gat	gag Glu gac	Glu gta Val acc	Leu aac Asn	Phe ggc Gly 25	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val 30	Ile 15 tcc Ser	ggc Gly	
35 40	atg Met 1 gtc Val gag Glu	gtg Val gag Glu ggc Gly acc	agc Ser Ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp	gta Val acc Thr	Leu aac Asn tac Tyr 40	Phe ggc Gly 25 ggc Gly ccc	Thr 10 cac His aag Lys	aag Lys ctg Leu	ttc Phe acc Thr	agc ser ctg Leu 45	gtg Val 30 aag Lys	Ile 15 tcc ser ctg Leu	ggc Gly atc Ile	96
35	atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala	gta Val acc Thr ccc Pro 55	Leu aac Asn tac Tyr 40 gtg Val	Phe ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr ccc	Val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser ctg Leu acc Thr	ggc Gly atc Ile acc Thr	96 144
35 40	atg Met 1 gtc Val gag Glu tgc Cys ctg Leu 65	gtg Val gag Glu ggc Gly acc Thr 50 ggc Gly	agc Ser ctg Leu gag Glu 35 acc Thr tac Tyr	aag Lys gac Asp 20 ggc Gly ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys ctg Leu	gag Glu gac Asp gcc Ala ctg Leu cag Gln	Glu gta Val acc Thr ccc Pro 55 tgc Cys	Leu aac Asn tac Tyr 40 gtg Val ttc Phe	Phe ggc Gly 25 ggc Gly ccc Pro gcc Ala	Thr 10 cac His aag Lys tgg Trp cgc Arg	Gly aag Lys ctg Leu ccc Pro tac Tyr 75 gaa	Val ttc Phe acc Thr acc Thr 60 ccc Pro	agc Ser ctg Leu 45 ctc Leu gac Asp	gtg Val 30 aag Lys gtg Val cac His	Ile 15 tcc Ser ctg Leu acc Thr	ggc Gly atc Ile acc Thr aag Lys 80 gag	96 144 192

				gac Asp											384
5				gac Asp											432
10				aac Asn 150											480
15				ttc Phe											522
20	ıs F1 ID N			L mut 7	atio	ons,	pos	. 1 N	Met 1	remov	red		•		
25				gag Glu										gtc Val	48
				gta Val											96
30				acc Thr											144
35				ccc Pro											192
40			-	tgc Cys 70		_	_			-		_	_		240
45				tcc Ser											288
				gac Asp											336
50				acc Thr											384
55				ggc Gly											432

												aag Lys			480
5					aag Lys										519
10				,M153 & 839	3⊤ mi Ə	ıtat:	ions								
15												ccc Pro			48
20												gtg Val 30			96
25												aag Lys			144
25												gtg Val			192
30												cac His			240
35	"yrd caa											gtc Val			288
40	_				_	-	_			_		cgc Arg 110	_		336
45												ctg Leu			384
43												ctg Leu			432
50			_						-		_	cag Gln			480
55					ttc Phe										522

Venus F1E, F46L, M153T mutations, pos. 1 Met removed SEQ ID NOS:840 & 841

5	gtg Val 1	agc Ser	aag Lys	ggc Gly	gag Glu 5	gag Glu	ctg Leu	ttc Phe	acc Thr	999 Gly 10	gtg Val	gtg Val	ccc Pro	atc Ile	ctg Leu 15	gtc Val	48
10						gta Val											96
15						acc Thr											144
						ccc Pro											192
20						tgc Cys 70											240
25						tcc Ser											288
30						gac Asp											336
35						acc Thr											384
						ggc Gly											432
40						gtc Val 150				_	_	_	_	_			480
45						aag Lys											519
50			LE, E			53T n	ıutat	ions									
<b>5</b> 5						gag Glu										ctg Leu	48

				gac Asp										96
5				gcc Ala										144
10				ctg Leu										192
15				cag Gln 70										240
20				aag Lys										288
				aag Lys									gag Glu	336
25				gac Asp										384
30				gac Asp										432
35				aac Asn 150										480
40		_		ttc Phe	_		_					_		522
45			764L 344 8	3T mi 5	ıtati	ions	, pos	s. 1	Met	remo	oved			
				gag Glu										48
50				gta Val										96
55				acc Thr										144

			aag Lys													·	192
5			ctg Leu	_	_		_	_			_		_	_			240
10			ttc Phe														288
15			ttc Phe 100														336
20			ggc Gly														384
			gag Glu												aac Asn		432
25			cac His														480
30			aac Asn		_		_					_					519
35			F64I 846 8			, V16	53A r	nutat	ions								
40			aag Lys					Phe									48
45		_	gac Asp 20		-	-				_		_					96
	Gly		ggc Gly														144
50			ggc Gly	_	_												192
55			ggc Gly														240

			-			aag Lys			-		_			_	_		288
5						aag Lys											336
10		_				gac Asp		_			_			_	_		384
15		_		_		gac Asp				_			_	_			432
20						aac Asn 150											480
			-	_		ttc Phe	_		-					_	-		522
25							•						•				
			1E, I	F64L,	M15	53T,	V163	3A mi	ıtat:	ions,	pos	s. 1	Met	remo	oved		
30 .			10S : 8														
30	gtg	agc	aag	ggc	gag	gag Glu											48
35	gtg Val 1 gag	agc Ser ctg	aag Lys gac	ggc Gly	gag Glu 5 gac	gag	Leu	Phe ggc	Thr	Gly 10 aag	Val	Val agc	Pro gtg	Ile tcc	Leu 15 ggc	Val gag	<b>48</b> 96
	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	Leu aac Asn	Phe ggc Gly ggc	Thr cac His 25	Gly 10 aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
35	gtg Val 1 gag Glu ggc Gly	agc Ser Ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	aac Asn tac Tyr	ggc Gly ggc Gly 40	Thr cac His 25 aag Lys	Gly 10 aag Lys ctg Leu	Val ttc Phe acc Thr	Val agc Ser ctg Leu ctc	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96
35 40 45	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	Leu aac Asn tac Tyr gtg Val 55	ggc Gly ggc Gly 40 ccc Pro	Thr cac His 25 aag Lys tgg Trp	Gly 10 aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr	Val agc ser ctg Leu ctc Leu 60 gac	gtg Val aag Lys 45 gtg Val	tcc Ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ctg Leu	96 144
35 40	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50 tac Tyr	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys ctg Leu	gag Glu 5 gac Asp gcc Ala ctg Leu cag Gln	gag Glu gta Val acc Thr ccc Pro	Leu aac Asn tac Tyr gtg Val 55 ttc Phe gcc	ggc Gly 40 ccc Pro	Thr cac His 25 aag Lys tgg Trp cgc Arg	Gly 10 aag Lys ctg Leu ccc Pro tac Tyr	Val ttc Phe acc Thr acc Thr	val agc Ser ctg Leu ctc Leu 60 gac Asp	gtg Val aag Lys 45 gtg Val cac	tcc Ser 30 ttc Phe acc Thr atg Met	Leu 15 ggc Gly atc Ile acc Thr aag Lys	yal gag Glu tgc Cys ctg Leu cgg Arg 80 cgc	96 144 192

5							aac Asn									884
·							ctg Leu								4	32
10							acc Thr								4	80
15							cac His								5	19
20			F46L 850 &		163A	muta	ation	ns								
25				 		_	ttc Phe							ctg · Leu		48
							ggc Gly 25									96
30				_			ggc Gly	_	_		_	_	_		1	44
35	tgc Cys						ccc Pro								1	92
40							gcc Ala								2	40
45							atg Met								2	88
				_	_	_	ggc Gly 105			_		_	_		3	36
50				_		_	gtg Val		-			_	_		3	84
55							atc Ile								4	32

					gtc Val											480
5					aag Lys											522
10			, M15 & 85		/163 <i>I</i>	A mut	tatio	ons,	pos	. 1 !	Met i	cemov	red			
15					ctg Leu											48
20					aac Asn											96
20					tac Tyr									tgc Cys		144
25		 _	_		gtg Val 55											192
30					ttc Phe											240
35					gcc Ala											288
40			_	_	gac Asp				_		_	_			•	336
40					ctg Leu											384
45					aac Asn 135											432
50					tat Tyr											480
55					atc Ile											519

Venus F1E, F46L,F64L,M153T,V163A mutations SEQ ID NOS:854 & 855

5	atg Met 1	gtg Val	agc Ser	aag Lys	ggc Gly 5	gag Glu	gag Glu	ctg Leu	ttc Phe	acc Thr 10	ggg Gly	gtg Val	gtg Val	ccc Pro	atc Ile 15	ctg Leu	48
10									ggc Gly 25								96
15									ggc Gly								144
									ccc Pro								192
20									gcc Ala								240
25									atg Met								288
30									ggc Gly 105								336
35									gtg Val								384
									atc Ile								432
40									atc Ile								480
45									cgc Arg								522
50				74.CT	TIC 4 I	M1.5	.am 1										
					F641 2 857		۷, ۳ د د	1163 <i>F</i>	A mut	atio	ns,	pos.	1 M	et r	remov	rea	
55	gtg Val 1	agc Ser	aag Lys	ggc Gly	gag Glu 5	gag Glu	ctg Leu	ttc Phe	acc Thr	999 Gly 10	gtg Val	gtg Val	ccc Pro	atc Ile	ctg Leu 15	gtc Val	48

						gta Val							96
5						acc Thr							144
10						ccc Pro							192
15						tgc Cys 70	Phe						240
20						tcc Ser							288
20						gac Asp						gtg Val	336
25						acc Thr							384
30						ggc Gly							432
35						gtc Val 150							480
40						aag Lys							519
	Veni	ıs Fi	IE. V	/163/	A muit	atio	חר					•	
45	SEQ	ID 1	10S:8	358 8	¥ 859	9							
						gag Glu							48
50						gac Asp							96
55						gcc Ala							144

			ggc Gly													192
5			ggc Gly													240
10			ttc Phe													288
15	-		ttc Phe 100		_	_	_				_		_	_		336
20			gag Glu													384
			aag Lys													432
25			agc Ser			_			_	_	-	_	_	_		480
30		_	gcc Ala			_		_				<b>-</b>	_			522
35			/163 <i>I</i> 360 8			on, p	pos.	1 Me	et re	emove	ed					
40			ggc Gly													48
45			ggc Gly 20													.96
70		 	gat Asp	_				_	_		_	_			_	144
50			aag Lys													192.
55			ctg Leu													240

				ttc Phe													288
5				ttc Phe 100													336
10				ggc Gly													384
15				gag Glu													432
20		Asn		cac His													480
				aac Asn										•			519
25																	
		ıs Fi		M1537			nutat	ions	5 .								
	024			002	. 00.	,											
30	atg	gtg	agc	aag Lys	ggc	gag											48
30 35	atg Met 1	gtg Val gag	agc Ser	aag Lys	ggc Gly 5	gag Glu gac	Glu gta	Leu	Phe ggc	Thr 10 cac	Gly	Val ttc	Val agc	Pro gtg	Ile 15 tcc	Leu	48 96
	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly 5 ggc Gly	gag Glu gac Asp	Glu gta Val acc	Leu aac Asn	Phe ggc Gly 25 ggc	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc ser	gtg Val 30	Ile 15 tcc ser	ggc Gly	
35 40	atg Met 1 gtc Val gag Glu	gtg Val gag Glu ggc Gly acc	agc Ser ctg Leu gag Glu 35	aag Lys gac Asp 20	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp gcc Ala	gta Val acc Thr	Leu aac Asn tac Tyr 40	Phe ggc Gly 25 ggc Gly ccc	Thr 10 cac His aag Lys	Gly aag Lys ctg Leu ccc	Val ttc Phe acc Thr	agc ser ctg Leu 45	gtg Val 30 aag Lys	Ile 15 tcc ser ttc Phe	Leu ggc Gly atc Ile	96
35	atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala ctg Leu	gta Val acc Thr  ccc Pro 55	Leu aac Asn tac Tyr 40 gtg Val	Phe ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	agc ser ctg Leu 45 ctc Leu	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser ttc Phe acc Thr	ggc Gly atc Ile acc Thr	96 144
35 40	atg Met 1 gtc Val gag Glu tgc Cys ttc Phe 65	gtg Val gag Glu ggc Gly acc Thr 50 ggc Gly	agc Ser ctg Leu gag Glu 35 acc Thr tac Tyr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys ctg Leu	gag Glu gac Asp gcc Ala ctg Leu cag Gln 70	gta Val acc Thr ccc Pro 55 tgc Cys	Leu aac Asn tac Tyr 40 gtg Val ttc Phe	Phe ggc Gly 25 ggc Gly ccc Pro gcc Ala atg	Thr 10 cac His aag Lys tgg Trp cgc Arg	Gly aag Lys ctg Leu ccc Pro tac Tyr 75 gaa	Val ttc Phe acc Thr acc Thr 60 ccc Pro	agc Ser ctg Leu 45 ctc Leu gac Asp	gtg Val 30 aag Lys gtg Val cac	Ile 15 tcc Ser ttc Phe acc Thr atg Met	ggc Gly atc Ile acc Thr aag Lys 80 gag	96 144 192

					acc Thr									3	384
5					ggc Gly 135										132
10					gtc Val									4	180
15					aag Lys									5	522
20	us F: ID 1				nutat	cions	s, po	os. :	l Met	ren	nove	f	•		
25					ctg Leu										48
					aac Asn										96
30	 	 _	_		tac Tyr		_	_		_	_		_	1	L44
35					gtg Val 55									1	192
40					ttc Phe									2	240
45					gcc Ala									2	88
40			_	_	gac Asp				_		_	_	 	3	336
50					ctg Leu									3	884
55					aac Asn 135									4	132

			_	cac His		_			_	_	_	_	_		 480
5				aac Asn											519
10	(YF		E co	et ad rresp 866 8	ponds	s to			175	-end	of :	YFP)			
15				gtg Val											48
20				ccc Pro 20											96
25				agc Ser											144
30				gtg Val											192
		aag Lys													198
35				03F r 868 8											
40				cag Gln			_					_		_	 48
45	_			gtg Val 20	_	_		_			_	_		_	96
50				aaa Lys											144
•				acc Thr											192
55	aag Lys 65														195

5				nutat k 87:		, Met	ado	ded (	g pos	s. 1						
ŭ													acc Thr		atc Ile	48
10													agc Ser 30			96
15													atg Met			144
20													gac Asp			192
25	tac Tyr 65	aag Lys														198
				mutat k 873												
30													ccc Pro			48
35	_			_	_		_				_	_	cac His 30	_		96
40													gtc Val			144
45													gag Glu			192
45	aag Lys 65															195
50				nutat k 875		Met	ado	ded @	) pos	s. 1						
55													acc Thr			48

											agc Ser 30		·	96
5											atg Met			144
10											gac Asp			192
15	tac Tyr 65	_												198
20		ıs F2		atio 7	on									
											ccc Pro	ggc Gly		48
25											tac Tyr 30			96
30											gtc Val			144
35	gag Glu										gag Glu			192
40°	aag Lys 65													195
45		ıs F2 ID N			on, N	1et a	addec	d @ p	os.	1				
-10											acc Thr			48
50											agc Ser 30			96
55											atg Met	ctg Leu		144

	ctg Leu			gcc Ala										192
5	tac Tyr 65	_									·			198
10	Venu SEQ				nutai	cions	3							
15	ggc Gly 1													48
20	gac Asp													96
20	gcc Ala												ctg Leu	144
25	gag Glu													192
30	aag Lys 65													195
35	Venu SEQ				muta	ation	ns, M	Met a	addeo	1 @ p	pos.	1		
40	atg Met 1													48
	ggc													96
45	tcc Ser													144
50	ctg Leu													192
55	tac Tyr 65													198

Venus F2E, S175G, Y203F mutations SEQ ID NOS:884 & 885

5															atc Ile 15		48
10															cag Gln		96
15															ctg Leu		144
20															ctg Leu		192
	aag Lys 65																195
25				31750 386 8			nutat	ions	s, Me	et ad	dded	@ po	os. i	L			
30															ccc Pro 15		48
35		_				_	_		_				_	_	ttc Phe	_	96
40															gtc Val		144
	_					_	_						_	_	gag Glu	_	192
45	tac Tyr 65	aag Lys															198
50	(YF	P F1E	CO	osit: cresp 388 8	onds	s to				1-19	91 of	E YFI	⊋)				
55															ctg Leu 15	gtc Val	48

							ggc Gly									96
5							ggc Gly 40								tgc Cys	144
10							ccc Pro									192
15			_	_	_		gcc Ala	_			_		_	_		240
20							atg Met									288
_0				_	_	_	ggc Gly			_		_	_			336
25							gtg Val 120									384
30 .							atc Ile									432
35		_			-		atc Ile	_	_	_	_	_	_			480
40							cgc Arg									528
40							cag Gln									570
45																
				ıtati 2 891						٠						
50							ctg Leu									48
55							aac Asn									96

				gcc Ala										144
5				ctg Leu										192
10				cag Gln 70										240
15		_		aag Lys		_	_		_		_	_		288
20				aag Lys										336
20				gac Asp										384
25				gac Asp				_		_	_			432
30				aac Asn 150										480
35	ggc Gly			ttc Phe										528
40				cac His										<b>573</b>
45			9R mi 392 8	ion,	pos.	. 1 N	1et 1	remov	red					٠
40				gag Glu										48
50				gta Val										96
<b>55</b>				acc Thr										144

						gtg Val 55							192
5						ttc Phe							240
10						gcc Ala							288
15						gac Asp							336
20						ctg Leu	Asn						384
	_	_		_		aac Asn 135	_		_	_		aac Asn	432
25						tat Tyr							480
30					Lys	atc Ile							528
35						cag Gln							570
40			5F mu 394 8										
45						gag Glu							48
40						gta Val							96
50						acc Thr							144
55						ccc Pro 55							192

					cag Gln 70											240
5					aag Lys											288
10	_				aag Lys	_	_				_		_	_		336
15					gac Asp											384
20	Ile				gac Asp											432
					aac Asn 150										aac Asn 160	480
25					ttc Phe											528
30					cac His											573
35			5F mi 396 8		ion, 7	pos	. 1 N	Met 1	cemov	ved						
40					gag Glu											48
45					gta Val											96
		 	_	_	acc Thr			_	_		_	_			_	144
50			_	_	ccc Pro											192.
55					tgc Cys 70											240

							gcc Ala										288
5							gac Asp									gtg Val	336
10							ctg Leu										384
15							aac Asn 135										432
20							tat Tyr										480
							atc Ile									gtg Val	528
25							cag Gln										570
30		F1F,							·						•		
35	SEQ atg	ID N	105:8	398 8 aag	ggc	gag	gag Glu										48
	sEQ atg Met 1	ID N gtg Val gag	agc ser ctg	aag Lys gac	ggc Gly 5 ggc	gag Glu gac		Leu aac	Phe ggc	Thr 10 cac	Gly aag	Val ttc	Val agc	Pro gtg	Ile 15 tcc	Leu	48 96
35 40	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly	gag Glu gac Asp	Glu gta	Leu aac Asn tac	ggc Gly 25	Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	gtg Val 30	Ile 15 tcc ser	Leu ggc Gly atc	
35	sEQ atg Met 1 gtc Val gag Glu tgc	gtg Val gag Glu ggc Gly	agc ser ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp gcc Ala	Glu gta Val acc	aac Asn tac Tyr 40	Phe ggc Gly 25 ggc Gly	Thr 10 cac His aag Lys	aag Lys ctg Leu	ttc Phe acc Thr	agc ser ctg Leu 45	gtg Val 30 aag Lys	11e 15 tcc ser ttc Phe	Leu ggc Gly atc Ile	96
35 40	sEQ atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc ser ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala ctg Leu	gta Val acc Thr	Leu aac Asn tac Tyr 40 gtg Val	Phe ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	11e 15 tcc ser ttc Phe acc Thr	ggc Gly atc Ile acc Thr	96

						aag Lys											336
5		_				gac Asp		_			_			_	_		384
10						gac Asp											432
15						aac Asn 150											480
20						ttc Phe											528
						cac His											573
25																	
		F1F,				ion,	pos.	. 1 1	1et 1	cemov	red						
						_											
30	gtg	agc	aag	ggc	gag	gag Glu											48
30 35	gtg Val 1	agc Ser	aag Lys gac	ggc gly	gag Glu 5 gac	gag	Leu aac	Phe ggc	Thr	Gly 10 aag	Val	Val agc	Pro gtg	Ile	Leu 15 ggc	Val gag	48 96
	gtg Val 1 gag Glu	agc Ser Ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	Leu aac Asn	Phe ggc Gly ggc	Thr cac His 25	Gly 10 aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
35 40	gtg Val 1 gag Glu ggc Gly	agc Ser Ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	Leu aac Asn tac Tyr	ggc Gly ggc Gly 40	Thr cac His 25 aag Lys	Gly 10 aag Lys ctg Leu	ttc Phe acc Thr	agc Ser ctg Leu	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96
35	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	Leu aac Asn tac Tyr gtg Val 55	ggc Gly 40 ccc Pro	Thr  cac His 25  aag Lys  tgg Trp  cgc	Gly 10 aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc	Val agc ser ctg Leu ctc Leu 60	gtg Val aag Lys 45 gtg Val	tcc ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	gag Glu tgc Cys ttc Phe	96 144
35 40	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50 tac Tyr	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys ctg Leu	gag Glu 5 gac Asp gcc Ala ctg Leu aag Lys	gag Glu gta Val acc Thr ccc Pro	Leu aac Asn tac Tyr gtg Val 55 ttc Phe gcc	ggc Gly 40 ccc Pro	Thr  Cac His 25  aag Lys  tgg Trp  cgc Arg	Gly 10 aag Lys ctg Leu ccc Pro tac Tyr	Val ttc Phe acc Thr acc Thr ccc Pro 75	Val agc Ser ctg Leu ctc Leu 60 gac Asp	gtg Val aag Lys 45 gtg Val cac	tcc Ser 30 ttc Phe acc Thr atg Met	Leu 15 ggc Gly atc Ile acc Thr aag Lys	yal gag Glu tgc Cys ttc Phe cgg Arg 80 cgc	96 144 192

	_		 	_	acc Thr	_		_		_	_			384
5					ggc Gly									432
10					gtc Val 150									480
15					aag Lys									528
20					tac Tyr									570
25		cine ID N			9 <b>M</b> mi 3	ıtati	ions			. •		٠.		
					gag Glu									48
30 .					gac Asp									96
35					gcc Ala									144
40					ctg Leu									192
45					atg Met 70									240
40					aag Lys									288
50	_				aag Lys	_	_		_		_	_		336
55					gac Asp									384

					gac Asp												432
5			. –		aac Asn 150	_			_	_	_	_	_	_			480
10					ttc Phe												528
15					cac His												573
20				3L,Q6 % 905	5 <b>9M</b> r	nutat	ions	s, po	os. 3	l Met	: rem	nove	i	·			
25					gag Glu										gtc Val		48
					gta Val												96
30					acc Thr												144
35	acc Thr				ccc Pro												192
40			_	_	tgc Cys 70		_	_			_		_	_		:	240
45					tcc Ser											:	288
<del>-1</del> 3					gac Asp											:	336
50					acc Thr											:	384
55					ggc Gly												432

										aag Lys		480
5	_			_		_			 _	ggc Gly	_	 528
10					cag Gln							570
15			1L mi 906 8									
20										ccc Pro		48
25										gtg Val 30		96
- 23										aag Lys		144
30										gtg Val		192
35										cac His		240
40		_		_		_	_	_		gtc Val	_	 288
AE										cgc Arg 110		336
45										ctg Leu		384
50										ctg Leu		432
55										cag Gln		480

					ttc Phe									528
5					cac His									573
10			4L mi 908 8		ion,	pos	. 1 N	Met 1	remo	red				
15					gag Glu									48
20					gta Val									96
25					acc Thr								tgc Cys	144
25					ccc Pro									192
30					tgc Cys 70									240
35	cac				tcc Ser									288
40				_	gac Asp	_				_	_	_	 	336
45	_			_	acc Thr	_			_		 _	_		384
<b>-</b>		Lys			ggc									432
50					gtc Val 150									480
55					aag Lys									528

						tac Tyr									570
5															
					S65T & 91:	, Y66V 1	√ mut	atio	ons						
10	_		_	_		gag Glu		_						_	48
15	_		_	_		gac Asp	_			_		_			96
20						gcc Ala					Thr				144
25	_				_	ctg Leu									192
						cag Gln 70									240
30						aag Lys									288
35						aag Lys									336
40						gac Asp									384
45		_		_		gac Asp				 		_	_		432
10						aac Asn 150									480
50						ttc Phe						_	_	 _	528
55						cac His									573

## CFP F1F, F64L,S65T,Y66W mutations, pos. 1 Met removed SEQ ID NOS:912 & 913

5				ctg Leu						48
10				aac Asn						96
15				tac Tyr						144
20				gtg Val 55						192
				ttc Phe					cgg Arg 80	240
25				gcc Ala						288
30				gac Asp						336
35	aag Lys			ctg Leu			 _	_		384
40				aac Asn 135						432
.0				tat Tyr						480
45				atc Ile						528
50				cag Gln						570

## CFP F1F, F64L,S65T,Y66W,N146I,M153T,V163A mutations SEQ ID NOS:914 & 915

5						gag Glu									48
10						gta Val									96
15						acc Thr									144
						ccc Pro 55									192
20						tgc Cys								aag Lys 80	240
25						tcc Ser									288
30	-				_	gac Asp	_			_		_	_		336
35						acc Thr									384
						ggc Gly 135									432
40			_			gtc Val			_	_	_	-	_		480
45						aag Lys									528
50		_	_	_		tac Tyr	_	_				~ ~	_		573

				S65T § 91		6W, I	N146	I, M:	153T	, V16	63A t	nutai	cions	s, po	os. 1	Met	remove
5													atc Ile				48
10													tcc Ser 30				96
15													ttc Phe				144
.0			_	_									acc Thr		-		192
20			_	_	_		-	_			_		atg Met	_			240
25													cag Gln				288
30				_	_					_		_	gcc Ala 110				336
35													aag Lys				384
		_		_				_			_	_	gag Glu				432
40		_			_				_	_	_	_	aag Lys				480
45													ggc Gly				528
50							cag Gln										570

## CFP F1F, Y66W mutation SEQ ID NOS:918 & 919

5					gag Glu										48
10	_	 _	_		gac Asp	_			_		_				96
15		 		-	gcc Ala			 _	_		_	_			144
.0	_			_	ctg Leu										192
20					cag Gln 70										240
25					aag Lys										288
30	_				aag Lys	_	_			_		_	_		336
35					gac Asp										384
555					gac Asp										432
40					aac Asn 150										480
45					ttc Phe										528
50					cac His										573

CFP F1F, Y66W mutation, pos. 1 Met removed SEQ ID NOS:920 & 921

5					ctg Leu								48
10					aac Asn								96
15					tac Tyr								144
					gtg Val 55								192
20					ttc Phe								240
25					gcc Ala								288
30			_	_	gac Asp			_	_	_			336
35					ctg Leu								384
					aac Asn 135								432
40					tat Tyr								480
45	_			_	atc Ile	_			 _		_		528
50					cag Gln								570

CFP F1F, Y66W, N146I mutations SEQ ID NOS:922 & 923

5						gag Glu										48
10						gta Val										96
15						acc Thr										144
	_			_	_	ccc Pro 55										192
20						tgc Cys									aag Lys 80	240
25		_			_	tcc Ser	_	_		_			_	_		288
30						gac Asp										336
35						acc Thr										384
			_	_		ggc Gly 135						_	_			432
40						gtc Val										480
45						aag Lys										528
50						tac Tyr										573
55				N146: & 925		atio	ons,	pos	. 1 1	Met 1	cemov	red				
						ctg Leu										48

	1			5				10			15		
5							cac His 25						96
10							aag Lys						144
							tgg Trp						192
15							cgc Arg						240
20							ccc Pro						288
25							aac Asn 105						336
30							aac Asn						384
50							ctg Leu						432
35							atg Met						480
40							cac His						528
45							aac Asn 185						570
50		F1F,	53T 926 8		ation 7	1							
							ttc Phe						48
55							ggc Gly 25						96

5						gcc Ala										144
	_				_	ctg Leu		_								192
10						cag Gln 70										240
15						aag Lys										288
20	-					aag Lys	_					_	-	_		336
25						gac Asp										384
20						gac Asp										432
30						aac Asn 150										480
35			_			ttc Phe	_		_				 _		_	528
40		_		_	_	cac His		_	_					_		573
45				53T m 928 8		ion,	pos	3. 1	Met	remo	oved	٠.				
						gag Glu										48
50						gta Val										96
55						acc Thr									tgc Cys	144

		 _	_	ccc Pro								192
5		 _	_	tgc Cys 70	_	_		_	_	_		240
10				tcc Ser								288
15				gac Asp								336
20				acc Thr								384
				ggc Gly								432
25				gtc Val 150								480
30				aag Lys								528
35				tac Tyr								570
40	F1F,											
45				gag Glu								48
				gac Asp								96
50				gcc Ala								144
55				ctg Leu								192

				cag Gln 70											240
5				aag Lys											288
10				aag Lys											336
15				gac Asp											384
20				gac Asp											432
				aac Asn 150											480
25				ttc Phe											528
30				cac His											573
35		, N14 NOS:9		Γ mut	tatio	ons,	pos	. 1 N	Met 1	remov	red				
40		_	 	gag Glu	_							-	_		48
45				gta Val											96
40				acc Thr						-	_		_		144
50				ccc Pro											192
55				tgc Cys 70										•	240

						tcc Ser											:	288
5					_	gac Asp					_		_	_				336
10						acc Thr											:	384
15						ggc											4	432
20						gtc Val 150											4	480
						aag Lys											į	528
25						tac Tyr									,		į	570
30 .		F1F				3T, V 5	/163/	A mi	ıtat:	ions								
30 .	SEQ atg	ID N	105:9	934 8 aag	935 ggc		gag	ctg	ttc	acc								48
	sEQ atg Met 1	ID N gtg Val gag	agc ser ctg	aag Lys Lys gac Asp	ggc Gly 5 ggc Gly	gag	gag Glu gta Val	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr 10 cac	Gly aag Lys	Val ttc Phe	Val agc Ser	Pro gtg	Ile 15 tcc	Leu		48 96
35 40	atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly gat	gag Glu gac Asp	gag Glu gta Val	ctg Leu aac Asn	ttc Phe ggc Gly 25	acc Thr 10 cac His	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	Pro gtg Val 30	Ile 15 tcc Ser	ggc Gly	-	
35	sEQ atg Met 1 gtc Val gag Glu tgc	gtg Val gag Glu ggc Gly	agc ser ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr 40	ttc Phe ggc Gly 25 ggc Gly	acc Thr 10 cac His aag Lys	aag Lys ctg Leu	Val ttc Phe acc Thr	agc ser ctg Leu 45	gtg Val 30 aag Lys	tcc ser ttc Phe	ggc Gly atc Ile acc		96
35 40	sEQ atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala	gag Glu gta Val acc Thr ccc Pro 55	ctg Leu aac Asn tac Tyr 40 gtg Val	ttc Phe ggc Gly 25 ggc Gly ccc Pro	acc Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc ser ttc Phe acc Thr	ggc Gly atc Ile acc Thr	:	96 144

						aag Lys											336
5						gac Asp											384
10						gac Asp											432
15						aac Asn 150											480
20						ttc Phe											528
20						cac His							Ile				573
25																	
		F1F				Γ,V16	53 <b>A</b> r	nutat	ions	s, po	os. 1	L Met	rer	nove	i		
				,50 (	. ,,	•											
30	gtg	agc	aag	ggc	gag	gag Glu											48
30 35	gtg Val 1	agc Ser	aag Lys gac	ggc gly	gag Glu 5 gac	gag Glu	Leu	Phe ggc	Thr	Gly 10 aag	Val	Val agc	Pro gtg	Ile	Leu 15 ggc	Val gag	48 96
	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta	Leu aac Asn	Phe ggc Gly	Thr cac His 25	Gly 10 aag Lys ctg	Val ttc Phe	Val agc Ser ctg	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
35 40	gtg Val 1 gag Glu ggc Gly	agc Ser Ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val	Leu aac Asn tac Tyr	ggc Gly ggc Gly 40	Thr cac His 25 aag Lys	Gly 10 aag Lys ctg Leu	ttc Phe acc Thr	agc ser ctg Leu	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96
35	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	Leu aac Asn tac Tyr gtg Val 55	Phe ggc Gly ggc Gly 40 ccc Pro	Thr cac His 25 aag Lys tgg Trp cgc	Gly 10 aag Lys ctg Leu ccc Pro	Val ttc- Phe acc Thr acc Thr	Val agc ser ctg Leu ctc Leu 60 gac	gtg Val aag Lys 45 gtg Val	tcc ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ttc Phe	96 144
35 40	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser ctg Leu gag Glu acc Thr 50 tac Tyr gac	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly 20 gat Asp aag Lys ctg Leu	gag Glu 5 gac Asp gcc Ala ctg Leu cag Gln	gag Glu gta Val acc Thr ccc Pro	Leu aac Asn tac Tyr gtg Val 55 ttc Phe gcc	Phe ggc Gly ggc Pro gcc Ala atg	Thr  cac His 25  aag Lys  tgg Trp  cgc Arg	Gly 10 aag Lys ctg Leu ccc Pro tac Tyr	Val ttc Phe acc Thr acc Thr ccc Pro 75	Val agc Ser ctg Leu ctc Leu 60 gac Asp	gtg Val aag Lys 45 gtg Val cac	tcc Ser 30 ttc Phe acc Thr atg Met	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ttc Phe cgg Arg 80 cgc	96 144 192

					acc Thr								384
5					ggc Gly								432
10					gtc Val 150								480
15					aag Lys								528
20					tac Tyr								570
25			5W,N: 938 8		, M153	3 <b>T</b> mi	ıtat:	ions					
					gag Glu								48
30					gac Asp								96
35					gcc Ala								144
40	_			_	ctg Leu								192
45					cag Gln 70								240
.0					aag Lys								288
50					aag Lys								336
55					gac Asp								384

							atc Ile								٠	432
5					_		atc Ile		_	_	_	_	_			480
10							cgc Arg									528
15							cag Gln 185									573
20	F1F				3T mı	ıtat	ions	, pos	s. 1	Met	remo	oved				
25	 _	_	 		_		acc Thr						_	_		48
20							cac His 25									96
30							aag Lys									144
35							tgg Trp									192
40							cgc Arg									240
45							ccc Pro									288
70							aac Asn 105		_		_	_				336
50							aac Asn				_	_				384
55				Gly			ctg Leu									432

			_			gtc Val 150				_	_	_	_	_			480
5						aag Lys										gtg Val	528
10						tac Tyr									·		570
15				5W, 1 942 8		I, M: 3	153T,	, V16	53 <b>A</b> r	mutai	cions	5					
20						gag Glu											48
25	_		_	_		gac Asp	_				_		_				96
					_	gcc Ala				_	_		_	_			144
30 .						ctg Leu											192
35						cag Gln 70											240
40						aag Lys											288
45						aag Lys											336
40						gac Asp											384
50		_		_		gac Asp				_			_	-			432
55						aac Asn 150											480

				aac Asn 165											528
5				gac Asp											573
10				N146: & 94!	L53T,	, V16	53A: r	nutat	ions	s, po	os. I	L Met	rer	moved	
15				gag Glu 5											48
20				gac Asp											96
25		 	_	gcc Ala			_	_		_	_			tgc Cys	144
				ctg Leu											192
30				cag Gln											240
35				aag Lys 85											288
40				aag Lys											336
45				gac Asp											3,84
40	_	Lys		gac Asp			_			_	_				432
50				aac Asn											480
55				ttc Phe 165											528

					cag Gln									570
5	F1F ID I	5A r 946 t	nuta § 94											
10					gag Glu									48
15					gta Val									96
20					acc Thr									144
					ccc Pro 55								acc Thr	192
25			_	_	tgc Cys		_	-		_			_	240
30 .					tcc Ser									288
35					gac Asp									336
40	_			_	acc Thr	_			_		_	_		384
					ggc Gly 135									432
45					gtc Val									480
50					aag Lys									528
55	 _	_	_		tac Tyr	_	_					_		573

## CFP F1F, S65A mutation, pos. 1 Met removed SEQ ID NOS:948 & 949

5	 	_		gag Glu 5		_							_	_		48
10				gac Asp												96
15	 		_	gcc Ala			 _	_		_	_			_		144
.0			_	ctg Leu												192
20				cag Gln												240
25				aag Lys 85												288
30				aag Lys	_	-			_		_	_			•	336
35				gac Asp												384
00			-	gac Asp						_	_					432
40				aac Asn												480
45				ttc Phe 165												528
50				cac His												570

## CFP F1F, S65A, Y66W, S72A mutations SEQ ID NOS:950 & 951

5							ccc Pro		48
10							gtg Val 30		96
15							aag Lys		144
.0							gtg Val		192
20							cac His		240
25							gtc Val		288
30							cgc Arg 110		336
35							ctg Leu		384
00							ctg Leu		432
40							cag Gln		480
45							gac Asp		528
50							ggc Gly 190		573

CFP F1F, S65A, Y66W, S72A mutations, pos. 1 Met removed SEQ ID NOS:952 & 953

5						ttc Phe					48
10		_	_	 _	-	ggc Gly	_	_		 	96
15						ggc Gly 40					144
						ccc Pro					192
20						gcc Ala					240
25						atg Met					288
30						ggc Gly					336
35						gtg Val 120					384
						atc Ile					432
40						atc Ile					480
45						cgc Arg					528
50	cag Gln					cag Gln					570

55

]

CFP F1F, S65A, Y66W, S72A, N146I, M153T, V163A mutations SEQ ID NOS:954 & 955

5													ccc Pro			48
10													gtg Val 30			96
15													aag Lys			144
													gtg Val			192
20		_		 _	_	_		_	_			_	cac His	_	aag Lys 80	240
25			_		_		_	_		_			gtc Val	_		288
30 .	_				_	_	_				_		cgc Arg 110	_		336
35													ctg Leu			384
33													ctg Leu			432
40													cag Gln			480
45													gac Asp			528
50													ggc Gly 190			573

	Met	rem	oved	¥66₩ & 95	2 <b>A</b> , 1	N146	I, M:	153T	, V1	63A i	muta <sup>,</sup>	tion	s, po	os. 1	
5					ctg Leu										48
10					aac Asn										96
15					tac Tyr			_		_	_			_	144
20					gtg Val 55										192
					ttc Phe										240
25					gcc Ala										288
30					gac Asp										336
35	aag Lys				ctg Leu										384
40					aac Asn 135										432
					tat Tyr										480
45					atc Ile										528
50					cag Gln										570

## BFP F1F, Y66H mutation SEQ ID NOS:958 & 959

5					gag Glu							ctg Leu	48
10					gta Val								96
15					acc Thr								144
.0					ccc Pro 55								192
20					tgc Cys								240
25		_		_	tcc Ser	_		_		_	_		288
30 .				_	gac Asp	_			_	_	_		336
35					acc Thr								384
00					ggc Gly 135								432
40					gtc Val								480
45					aag Lys								528
50					tac Tyr								573

BFP F1F, Y66H mutation, pos. 1 Met removed SEQ ID NOS:960 & 961

5				gag Glu									48
10				gta Val									96
15				acc Thr									144
				ccc Pro									192
20		 _	_	tgc Cys 70		_	_			_	_		240
25				tcc Ser									288
30			_	gac Asp	_			_	_	_			336
35				acc Thr									384
				ggc Gly									432
40				gtc Val 150									480
45				aag Lys									528
50				tac Tyr									570
		1L,Y6 962 8		nutat 3	ions	3							
55				gag Glu								· · .	48

5				gac Asp										g	96
	 	 	_	gcc Ala				_	_		_			14	14.
10				ctg Leu										19	92
15				cag Gln 70										24	<del>1</del> 0
20				aag Lys										28	38
25				aag Lys										33	36
				gac Asp										38	34
30				gac Asp										43	32
35		_		aac Asn 150	-			_	_	_	_	_	_	4.8	30
40				ttc Phe										52	28
45				cac His										57	73
											_				
50		1L, Y6 964 8		nutat 5	cions	s, po	os. I	L Met	: rem	noved					
				gag Glu										. 4	18
55				gta Val										S	96

5							ggc Gly 40									144
							ccc Pro									192
10			_	_	_		gcc Ala	_			_		_	_		240
15							atg Met						Gln			288
20				-		_	ggc Gly			_		_	_			336
25							gtg Val 120								atc Ile	384
							atc Ile									432
30							atc Ile									480
35							cgc Arg									528
40							cag Gln									570
45		, F64 NOS:9				15F n	nutat	ions	5		٠.					
							ctg Leu									48
50							aac Asn								ggc Gly	96
55							tac Tyr 40								atc Ile	144

				ctg Leu											192
5				cag Gln 70											240
10				aag Lys											288
15	_			aag Lys	_	_				_		_	_		336
20				gac Asp			Val								384
20		_	_	 gac Asp				-			_	_			432
25			_	aac Asn 150	_			_	_	_	_	_			480
30 .				ttc Phe											528
35				cac His											573
40			1L, 1 968 &	, Y14 9	15F n	nutat	cions	s, po	os. 1	L Met	: ren	nove	i		
45				gag Glu											48
70				gta Val											96
50				acc Thr											144
55				ccc Pro											192

,		 _	_	_	gcc Ala	_			_		_	-		240
5					atg Met									288
10					ggc Gly									336
15					gtg Val 120									384
20					atc Ile									432
					atc Ile									480
25					cgc Arg									528
30					cag Gln									570
35	F1F,													
40					ctg Leu									48
45					aac Asn									96
40	 	 	_	_	tac Tyr 40		_	_		_	_			144
50					gtg Val									192
55					ttc Phe									240

					ttc Phe 85													288
5					ttc Phe											gag Glu		336
10					ggc Gly													384
15					gag Glu	Asp												432
20					cac His													480
					aac Asn 165													528
25					gac Asp													573
30					mutat § 973		, pos	s. 1	Met	remo	oved							
30	SEQ gtg	ID Nagc	NOS:9	972 8 ggc		3 gag	ctg	ttc	acc	<b>3</b> 99	gtg							48
	SEQ gtg Val 1 gag	agc Ser	aag Lys gac	ggc Gly ggc	gag Glu	gag Glu gta	ctg Leu	ttc Phe ggc	acc Thr	ggg Gly 10 aag	gtg Val ttc	Val agc	Pro gtg	Ile tcc	Leu 15 ggc	Val gag	·	48 96
35	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20 gat	gag Glu 5 gac	gag Glu gta Val	ctg Leu aac Asn	ttc Phe ggc Gly	acc Thr cac His 25	ggg Gly 10 aag Lys	gtg Val ttc Phe	Val agc Ser	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc		
35 40	gtg Val 1 gag Glu ggc Gly	agc Ser ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr	ttc Phe ggc Gly ggc Gly 40 ccc	acc Thr cac His 25 aag Lys	ggg Gly 10 aag Lys ctg Leu	gtg Val ttc Phe acc Thr	agc ser ctg Leu	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys		96
35 40	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser ctg Leu gag Glu acc Thr 50 tac	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr gtg Val 55	ttc Phe ggc Gly ggc Gly 40 ccc Pro	acc Thr cac His 25 aag Lys tgg Trp	ggg Gly 10 aag Lys ctg Leu ccc Pro	gtg Val ttc Phe acc Thr	Val agc ser ctg Leu ctc Leu 60 gac	Pro gtg Val aag Lys 45 gtg Val cac	tcc Ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ttc Phe		96 144

										tac Tyr							336
5										cgc Arg							384
10										ggg Gly							432
15										gcc Ala							480
20										aac Asn 170							528
										acc Thr							570
25																	
				F46L, 974 8			11537	r, V:	163A	muta	ation	ıs					
30										acc Thr 10							48
30 35	Met 1 gtc	Val gag	Ser ctg	Lys gac	Gly 5 ggc	Glu gac	Glu gta	Leu	Phe ggc	Thr	Gly aag	Val ttc	Val agc	Pro gtg	Ile 15 tcc	Leu ggc	48 96
	Met 1 gtc Val gag	Val gag Glu ggc	Ser ctg Leu gag	Lys gac Asp 20	Gly 5 ggc Gly gat	Glu gac Asp	Glu gta Val acc	Leu aac Asn	Phe ggc Gly 25 ggc	Thr 10 cac	Gly aag Lys ctg	Val ttc Phe	Val agc Ser	gtg Val 30	Ile 15 tcc Ser	Leu ggc Gly atc	
35 40	Met 1 gtc Val gag Glu	yal gag Glu ggc Gly	ctg Leu gag Glu 35	gac Asp 20 ggc Gly	Gly 5 ggc Gly gat Asp	gac Asp gcc Ala	Glu gta Val acc Thr	aac Asn tac Tyr 40	ggc Gly 25 ggc Gly	Thr 10 cac His	aag Lys ctg Leu	ttc Phe acc Thr	agc Ser ctg Leu 45	gtg Val 30 aag Lys	lle 15 tcc Ser ctg Leu	Leu ggc Gly atc Ile	96
35	Met 1 gtc Val gag Glu tgc Cys	yal gag Glu ggc Gly acc Thr 50	ser ctg Leu gag Glu 35 acc Thr	gac Asp 20 ggc Gly ggc	Gly 5 ggc Gly gat Asp aag Lys	gac Asp gcc Ala ctg Leu	gta Val acc Thr ccc Pro 55	Leu aac Asn tac Tyr 40 gtg Val	ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys	Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	Val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser ctg Leu acc Thr	Leu ggc Gly atc Ile acc Thr	96 144
35 40	Met 1 gtc Val gag Glu tgc Cys ctg Leu 65 cgg	yal gag Glu ggc Gly acc Thr 50 ggc Gly	ser ctg Leu gag Glu 35 acc Thr tac Tyr	gac Asp 20 ggc Gly ggc Gly	Gly 5 ggc Gly gat Asp aag Lys ctg Leu ttc	gac Asp gcc Ala ctg Leu cag Gln 70	Glu gta Val acc Thr ccc Pro 55 tgc Cys	Leu aac Asn tac Tyr 40 gtg Val ttc Phe	ggc Gly 25 ggc Gly ccc Pro	Thr 10 cac His aag Lys tgg Trp	Gly aag Lys ctg Leu ccc Pro tac Tyr 75 gaa	Val ttc Phe acc Thr acc Thr 60 ccc Pro	Val agc Ser ctg Leu 45 ctc Leu gac Asp	gtg Val 30 aag Lys gtg Val cac	Ile 15 tcc Ser ctg Leu acc Thr	ggc Gly atc Ile acc Thr aag Lys 80 gag	96 144 192

				gac Asp											384
5				gac Asp											432
10		_		aac Asn 150	-				_	_	_	_	_		480
15				ttc Phe											528
20				cac His											573
25		F46L, 976 8			11537	Γ,V16	53 <b>A</b> r	nutat	ions	s, po	os. 1	L Met	ren	noved	
				gag Glu											48
30 .				gta Val											96
35				acc Thr											144
40		 _	_	ccc Pro										_	192
45				tgc Cys 70											240
				tcc Ser											288
50				gac Asp											336
55				acc Thr											384

				aac Asn 135					4	132
5				tat Tyr					. 4	80
10				atc Ile					5	528
15				cag Gln					5	570
20	ıs Fl ID N			on						
25				gag Glu						48
30				gta Val						96
30				acc Thr					1	.44
35				ccc Pro 55					1	.92
40				tgc Cys					2	40
45				tcc Ser					Ź	88
				gac Asp					3	36
50				acc Thr					. 3	84
55				ggc Gly 135					4	32

5								tat Tyr										480
								atc Ile										528
10								cag Gln										573
15				V1637 980 8			on, p	pos.	1 Me	et re	emove	ed						
20								ttc Phe										48
25								ggc Gly								gag Glu		96
								ggc Gly 40										144
30								ccc Pro										192
35	ggc Gly 65							gcc Ala										240
40								atg Met										288
45								ggc Gly										3,36
								gtg Val 120										384
50								atc Ile										432
55	tac Tyr 145	aac Asn	agc Ser	cac His	aac Asn	gtc Val 150	tat Tyr	atc Ile	atg Met	gcc Ala	gac Asp 155	aag Lys	cag Gln	aag Lys	aac Asn	ggc Gly 160	•	480

					aag Lys									528
5					tac Tyr									570
10				Γ,V16 Σ 983	53 <b>A</b> m	nutat	cions	5						
15					gag Glu									48
20	_	 _	_		gac Asp	_				_	_			96
25		 		_	gcc Ala				_	_	_	_		144
20					ctg Leu									192
30					cag Gln 70									240
35					aag Lys									288
40					aag Lys									336
45					gac Asp									384
					gac Asp									432
50					aac Asn 150									480
55					ttc Phe									528

					cag Gln									573
5		M153' 984 (		nutai	cions	3, pa	os. :	1 Met	c rem	nove	i			
10					ttc Phe									48
15					ggc Gly									96
20					ggc Gly 40									144
20					ccc Pro							ttc Phe		192
25					gcc Ala									240
30					atg Met								•	288
35					ggc Gly									336
40					gtg Val 120									384
10			_		atc Ile	_			_	_				432
45					atc Ile									480
50					cgc Arg									528
55					cag Gln									570

Venus F1F, S175G mutation SEQ ID NOS:986 & 987

5				gag Glu										48	}
10				gac Asp										96	5
15	 	 	_	gcc Ala				_	_		_	_		144	F
				ctg Leu										192	?
20				cag Gln 70	Cys									240	)
25				aag Lys										288	3
30				aag Lys										336	5
35				gac Asp										384	ŧ
				gac Asp										432	}
40		_		aac Asn 150	_								_	480	)
45				ttc Phe										528	3
50				cac His										573	3
			3 mut \$ 989	tatio	on, p	os.	1 Me	et re	emove	ed					
55				gag Glu										4.8	3

5											ttc Phe					96
											acc Thr					144
10											acc Thr					192
15											ccc Pro 75					240
20											ggc Gly					288
25											aag Lys				gtg Val	336
											atc Ile					384
30											cac His					432
35	tac Tyr 145										gac Asp 155					480
40											atc Ile					528
45	cag Gln	ctc Leu	gcc Ala	gac Asp 180	cac His	tac Tyr	cag Gln	cag Gln	aac Asn 185	acc Thr	ccc Pro	atc Ile	ggc Gly	gac Asp 190		570
			F, N 10s:9				nutat	ions	3							
50											999 Gly					48
55											aag Lys					96

				gcc Ala											144
5			_	ctg Leu											192
10				cag Gln 70											240
15				aag Lys											288
20				aag Lys											336
				gac Asp										ggc Gly	384
25				gac Asp											432
30				aac Asn 150											480
35				ttc Phe											528
40	 _	_	_	cac His		_	_						_		573
45			Γ,S17 2 993	75G n	nutat	ions	s, po	os. 1	L Met	rem	noved	i			
				gag Glu											48
50				gta Val											96
55				acc Thr											144

								ccc Pro									192
5						-		gcc Ala				_		_	_		240
10								atg Met									288
15					_	_	_	ggc Gly			_		_	_			336
20								gtg Val 120									384
20								atc Ile								aac Asn	432
25								atc Ile									480
30								cgc Arg									528
35	cag Gln							cag Gln									570
	Veni	ıs Fi	lF, V	/163/	A,S1	75G r	nutat	ions	3								
40				994 8 aaq			gag	ctg	ttc	acc	aaa	ata	ata	ccc	atc	cta	48
45								Leu									
								aac Asn									96
50								tac Tyr 40									144
55						Leu		gtg Val									192

				cag Gln 70											240
5				aag Lys										gag Glu	288
10	_			aag Lys	_	_				_		_	_		336
15				gac Asp											384
20				gac Asp											432
				aac Asn 150										aac Asn 160	480
25				ttc Phe											528
30				cac His											573
35		ıs F: ID 1		175G 997	muta	ation	ns, p	os.	1 Me	et re	emove	ed			
40				gag Glu											48
45				gta Val											96
				acc Thr											144
50				ccc Pro											192
55				tgc Cys 70											240

										gaa Glu 90							288
5										tac Tyr							336
10										cgc Arg							384
15										ggg Gly							432
20		Asn								gcc Ala							480
										aac Asn 170							528
25										acc Thr							570
30				M1537 998 8			, S1	75G r	nutat	cions	5 .						
30 35	SEQ atg	ID I	10S:9	998 8 aag	ggc	gag	gag	ctg	ttc		ggg						48
	sEQ atg Met 1	ID Material States of the stat	agc ser ctg	aag Lys gac	ggc Gly 5 ggc	gag Glu gac	gag Glu gta	ctg Leu aac	ttc Phe ggc	acc Thr	ggg Gly aag	Val	Val	Pro gtg	Ile 15 tcc	Leu ggc	48 96
35 40	sEQ atg Met 1 gtc Val	gtg Val gag Glu	agc Ser ctg Leu	aag Lys gac Asp 20	ggc Gly 5 ggc Gly	gag Glu gac Asp	gag Glu gta Val	ctg Leu aac Asn	ttc Phe ggc Gly 25	acc Thr 10	ggg Gly aag Lys	Val ttc Phe	Val agc Ser	gtg Val 30	Ile 15 tcc Ser	ggc Gly	
35	sEQ atg Met 1 gtc Val gag Glu tgc	gtg Val gag Glu ggc Gly acc	agc ser ctg Leu gag Glu 35	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp	gag Glu gac Asp gcc Ala	gag Glu gta Val acc Thr	ctg Leu aac Asn tac Tyr 40	ttc Phe ggc Gly 25 ggc Gly	acc Thr 10 cac His	ggg Gly aag Lys ctg Leu	Val ttc Phe acc Thr	Val agc Ser ctg Leu 45	gtg Val 30 aag Lys	Ile 15 tcc ser ttc Phe	Leu ggc Gly atc Ile	96
35 40	sEQ atg Met 1 gtc Val gag Glu tgc Cys	gtg Val gag Glu ggc Gly acc Thr 50	agc Ser Ctg Leu gag Glu 35 acc Thr	aag Lys gac Asp 20 ggc Gly	ggc Gly 5 ggc Gly gat Asp aag Lys	gag Glu gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr ccc Pro 55	ctg Leu aac Asn tac Tyr 40 gtg Val	ttc Phe ggc Gly 25 ggc Gly ccc Pro	acc Thr 10 cac His aag Lys	ggg Gly aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr 60	Val agc ser ctg Leu 45 ctc Leu gac	gtg Val 30 aag Lys gtg Val	Ile 15 tcc Ser ttc Phe acc Thr	Leu ggc Gly atc Ile acc Thr	96 144

										aac Asn							336
5										aac Asn							384
10										ctg Leu							432
15				_			Val			acc Thr	_	_	_	_	_		480
20										cac His 170							528
20										aac Asn							573
25																	
		us Fi					S175	5G mi	ıtat	ions,	pos.	. 1 1	Met 1	cemov	red		
	-				u 1,	301											
30	gtg	agc	aag	ggc	gag	gag				999 Gly 10							48
35	gtg Val 1	agc Ser ctg	aag Lys gac	ggc Gly	gag Glu 5 gac	gag Glu gta	Leu aac	Phe ggc	Thr	Gly	Val ttc	Val	Pro gtg	Ile	Leu 15 ggc	Val gag	48 96
	gtg Val 1 gag Glu	agc Ser ctg Leu	aag Lys gac Asp	ggc Gly ggc Gly 20	gag Glu 5 gac Asp	gag Glu gta Val	Leu aac Asn	Phe ggc Gly ggc	Thr cac His 25	Gly 10 aag	Val ttc Phe	Val agc Ser	Pro gtg Val aag	tcc ser 30	Leu 15 ggc Gly atc	Val gag Glu tgc	
35	gtg Val 1 gag Glu ggc Gly	agc Ser ctg Leu gag Glu	aag Lys gac Asp ggc Gly 35	ggc Gly ggc Gly 20 gat Asp	gag Glu 5 gac Asp gcc Ala	gag Glu gta Val acc Thr	aac Asn tac Tyr	ggc Gly ggc Gly 40	Thr cac His 25 aag Lys	Gly 10 aag Lys ctg	ttc Phe acc Thr	val agc ser ctg Leu ctc	gtg Val aag Lys 45	tcc ser 30 ttc Phe	Leu 15 ggc Gly atc Ile	yal gag Glu tgc Cys	96
35	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser ctg Leu gag Glu acc Thr 50	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly 20 gat Asp aag Lys	gag Glu 5 gac Asp gcc Ala ctg Leu	gag Glu gta Val acc Thr	Leu aac Asn tac Tyr gtg Val 55 ttc	ggc Gly ggc Gly 40 ccc Pro	Thr cac His 25 aag Lys tgg Trp cgc	Gly 10 aag Lys ctg Leu	Val ttc Phe acc Thr acc	Val agc Ser ctg Leu ctc Leu 60	gtg Val aag Lys 45 gtg Val	tcc Ser 30 ttc Phe acc Thr	Leu 15 ggc Gly atc Ile acc Thr	yal gag Glu tgc Cys ttc Phe	96 144
35	gtg Val 1 gag Glu ggc Gly acc Thr	agc Ser Ctg Leu gag Glu acc Thr 50 tac Tyr	aag Lys gac Asp ggc Gly 35 ggc Gly	ggc Gly ggc Gly 20 gat Asp aag Lys ctg Leu	gag Glu 5 gac Asp gcc Ala ctg Leu cag Gln	gag Glu gta Val acc Thr ccc Pro	Leu aac Asn tac Tyr gtg Val 55 ttc Phe gcc	ggc Gly 40 ccc Pro	Thr  cac His 25  aag Lys  tgg Trp  cgc Arg	Gly 10 aag Lys ctg Leu ccc Pro	Val ttc Phe acc Thr acc Thr ccc Pro 75	val agc ser ctg Leu ctc Leu 60 gac Asp	gtg Val aag Lys 45 gtg Val cac	tcc Ser 30 ttc Phe acc Thr atg Met	Leu 15 ggc Gly atc Ile acc Thr aag Lys	yal gag Glu tgc Cys ttc Phe cgg Arg 80 cgc	96 144 192

				acc Thr										384
5		Lys		ggc Gly										432
10				gtc Val 150										480
15				aag Lys										528
20				tac Tyr										570
						,								
25			F46L, 1002	4L, N 003	M1537	r, V:	163A,	, S17	75G n	nutat	ions	3		
				gag Glu										48
30				gac Asp										96
35	gag Glu			gcc Ala										144
40				ctg Leu										192
45				cag Gln 70										240
70				aag Lys										288
50				aag Lys										336
55				gac Asp										384

						ggc Gly 135										432
5						gtc Val										480
10		_	_			aag Lys		_					_			528
15						tac Tyr										573
20	as Fi ID 1					53T,\	/163/	A, S17	75G 1	nutat	ions	s, po	os. I	L Met	removed	
25						ctg Leu										48
						aac Asn										96
30						tac Tyr										144
35			_	_		gtg Val 55									_	192
40			_	_	_	ttc Phe	_	_			_		_	_		240
45						gcc Ala										288
				_	_	gac Asp				_		-	_			336
50						ctg Leu										384
55						aac Asn 135										432

					aac Asn											480
5					ttc Phe 165											528
10					cac His											570
15	(YFI	P F21	CO1	cresp	dded oonds & 10	s to			lues	192	-end	of Y	YFP)	·		
20					ctg Leu 5											48
25					gac Asp											96
					gcc Ala											144
30	aag Lys		÷					•								147
35					nutat & 10											
40				Leu	ctg Leu 5											48
45					ccc Pro											96
					gcc Ala											144
50					nutat & 10		Met	: add	led @	) pos	5.1					
55					ctg Leu 5											48

										cgc Arg							96
5										ctc Leu							144
10	aag Lys																147
15				03H r 1012													
										tac Tyr 10							48
20										gat Asp						gag Glu	96
25										ggc Gly							144
30				03H r 1014			Met	ado	ded (	oq @	3. 1						
35										cac His 10							48
00	_	_	-		_				_	cgc Arg	_		_	_	_	_	96
40					_	_				ctc Leu		_	_		_		144
45	aag Lys																147
50				03T r 1016													
										tac Tyr 10							48
55										gat Asp							96

5				gcc Ala													144
				03T 1 1018			, Mei	t add	ded (	g pos	s. 1						
10				gtg Val													48
15				aaa Lys 20													96
20	gag Glu	ttc Phe	gtg Val 35	acc Thr	gcc Ala	gcc Ala	ggg Gly	atc Ile 40	act Thr	ctc Leu	ggc Gly	atg Met	gac Asp 45	gag Glu	ctg Leu	tac Tyr	144
	aag Lys																147
25	(RFI	P F17	A CO	s. 1 rres <sub>l</sub> 1020	onds	s to		resid	dues	1-39	of	mRFI	P)				
30				gag Glu													48
35	atg Met			tcc Ser 20													96
40				ccc Pro													114
45	(RFI	F2 <i>I</i>	ooi	et ac rresp 1022	onds	s to			lues	40-€	end c	of mF	RFP)				·
50				cag Gln													48
				tgg Trp 20													96
55				aag Lys													144

5					gag Glu								192
Ū					gac Asp							ttc Phe 80	240
10					ggc Gly								288
15					ggc								336
20					aag Lys 120				Lys				384
25					gac Asp								432
					ccc Pro								480
30					gag Glu								528
35					tcc Ser								561
40	(RF	3 CO	ond		i resid	dues	1-10	00 of	f mRI	FP)			
45					aag Lys								48
50					cac His								96
- •					acc Thr 40								144
55					gcc Ala								192

5					_	_		gtg Val	_			_	_			_	240
								gag Glu									288
10			gag Glu					•									300
15	(RFI	P F2I	, Met 3 coi NOS:1	resp	onds	s to		resio	dues	102	-225	(end)	of	mRFI	?)		
20								acc Thr									48
25					_		_	ctg Leu	_							gac Asp	96
								acc Thr 40									144
30								gcc Ala									192
35								cac His									240
40								cag Gln									288
45								cac His									336
							Gly	cgc Arg 120	His								375
50	(RFI	P F10	, pos C cos NOS:	resp	onds	s to		resio	lues	1-11	L5 of	E mRI	?P)				
55								aag Lys									48

5							ggc Gly										96
							ggc Gly										144.
10							ttc Phe 55										192
15							tac Tyr										240
20							ccc Pro										288
25							gtg Val										336
		gac Asp															342
30 .	(RF	P F20	C CO1	cresp			3. 1 aa 1	resid	dues	116-	-225	(end)	of	mRFI	?)		
35	(RF) SEQ atg	P F20 ID 1 ggc	C CO1 10S:	rresp 1030 ttc	eonds & 10 atc	tac		gtg	aag	ctg	cgc	ggc	acc	aac	ttc		48
	(RF) SEQ atg Met 1	P F20 ID 1 ggc Gly gac	gag Glu	ttc Phe	atc Ile 5	tac Tyr	aa 1	gtg Val aag	aag Lys aag	ctg Leu 10	cgc Arg	ggc ggc	acc Thr	aac Asn gag	ttc Phe 15 gcc	Pro	48
35	(RF) SEQ atg Met 1 tcc Ser	P F20 ID 1 ggc Gly gac Asp	gag Glu ggc Gly	ttc Phe ccc Pro 20	atc Ile 5 gta Val	tac Tyr atg Met	aag Lys cag	gtg Val aag Lys	aag Lys aag Lys 25	ctg Leu 10 acc Thr	cgc Arg atg Met	ggc Gly ggc Gly	acc Thr tgg Trp	aac Asn gag Glu 30	ttc Phe 15 gcc Ala	tcc ser aag	
35 40 45	(RF) SEQ atg Met 1 tcc Ser acc Thr	P F20 ID 1 ggc Gly gac Asp gag Glu	gag Glu ggc Gly cgg Arg 35	ttc Phe ccc Pro 20 atg Met	atc Ile 5 gta Val tac Tyr	tac Tyr atg Met ccc Pro	aag Lys cag Gln	gtg Val aag Lys gac Asp 40	aag Lys aag Lys 25 ggc Gly	ctg Leu 10 acc Thr gcc Ala	cgc Arg atg Met ctg Leu	ggc Gly ggc Gly aag Lys	acc Thr tgg Trp ggc Gly 45	aac Asn gag Glu 30 gag Glu	ttc Phe 15 gcc Ala atc Ile	tcc ser aag Lys	96
35	(RF) SEQ atg Met 1 tcc Ser acc Thr atg Met	ggc Gly gac Asp gag Glu agg Arg 50 acc	gag Glu ggc Gly cgg Arg 35 ctg Leu	ttc Phe ccc Pro 20 atg Met aag Lys	ecceptorial property of the content	tac Tyr atg Met ccc Pro aag Lys	aa i aag Lys cag Gln gag Glu gac Asp	gtg Val aag Lys gac Asp 40 ggc Gly	aag Lys aag Lys 25 ggc Gly ggc	ctg Leu 10 acc Thr gcc Ala cac	cgc Arg atg Met ctg Leu tac Tyr	ggc Gly ggc Gly aag Lys gac Asp 60 ccc	acc Thr tgg Trp ggc Gly 45 gcc Ala	aac Asn gag Glu 30 gag Glu gag Glu	ttc Phe 15 gcc Ala atc Ile gtc Val	tcc ser aag Lys aag	96 144

5			cag Gln														333
10	(RF	P F1I	, pos D cos NOS:	rresp	ponds	s to		resio	dues	1-15	53 of	E mRI	FP)				
			tcc Ser														48
15			ggc Gly														96
20			cgc Arg 35													:	144
25			ggc Gly														192
30			ggc Gly														240
			aag Lys													:	288
35			gag Glu													:	336
40		Asp	ggc Gly 115	Glu		Ile	Tyr		Val							:	384
45			gac Asp													•	432
50			gag Glu													•	456
	(RFI	P F2I	, Met D cor NOS:1	resp	onds	to		resid	lues	154-	-225 (	(end)	of	mRFI	?)		
55			ggc Gly														48

5	-	 			_			gtc Val 25	_					 	96
								tac Tyr							144
10								acc Thr							192
15					tcc Ser 70										219
20	(RF	E CO	rresp	onds			resio	dues	1-16	59 of	f mRI	P)			
25								gag Glu							48
30								gag Glu 25							96
								cag Gln							144
35								tgg Trp							192
40				_	_			aag Lys			_	_		_	240
45								ggc Gly							288
50								acc Thr 105							336
-								gtg Val					Thr		384
55		_			_	_		aag Lys						 _	432

5						tac Tyr 150											480
ŭ	_	_		_	_	ctg Leu	_	_									504
10	(RFF	F21	CO	resp		pos to 039		resid	dues	170-	-225	(end)	of	mRFI	· P)		
15						gac Asp											48
20	_			_	_	ccc Pro		_		_		_		_	_	_	96
25						gag Glu											144
						tcc Ser											171
30	(RFF	P F1E	e c	orres		remo ds to 041		res	idues	s 1-3	L84 d	of mI	RFP)				
30 35	(RFF SEQ gcc	F1F ID N	F co NOS:	orres 1040 gag	spond & 10 gac	is to 041	atc	aag	gag	ttc	atg	cgc	ttc				48
	(RFF SEQ gcc Ala 1	F1F ID N tcc Ser	tcc ser	gag Glu tcc	spond & 10 gac Asp 5 gtg	is to 041 gtc	atc Ile	aag Lys cac	gag Glu gag	ttc Phe 10	atg Met gag	cgc Arg	ttc Phe gag	Lys	Val 15 gag	Arg	48 96
35	gcc Ala 1 atg Met	tcc Ser gag Glu	tcc ser ggc Gly	gag Glu tcc Ser 20	gac Asp 5 gtg Val	ds to 041 gtc Val aac	atc Ile ggc Gly	aag Lys cac His	gag Glu gag Glu 25 cag	ttc Phe 10 ttc Phe	atg Met gag Glu	cgc Arg atc Ile	ttc Phe gag Glu ctg	Lys ggc Gly 30 aag	Val 15 gag Glu gtg	Arg ggc Gly acc	
35 40 45	gcc Ala 1 atg Met gag Glu	tcc Ser gag Glu ggc Gly	tcc ser ggc Gly cgc Arg 35	gag Glu tcc Ser 20 ccc Pro	gac Asp 5 gtg Val tac Tyr	gtc Val aac Asn	atc Ile ggc Gly ggc Gly	aag Lys cac His acc Thr 40	gag Glu gag Glu 25 cag Gln	ttc Phe 10 ttc Phe acc Thr	atg Met gag Glu gcc Ala	cgc Arg atc Ile aag Lys	ttc Phe gag Glu ctg Leu 45	ggc Gly 30 aag Lys	Val 15 gag Glu gtg Val	Arg ggc Gly acc Thr	96
35 40	gcc Ala 1 atg Met gag Glu aag Lys	tcc Ser gag Glu ggc Gly ggc Gly tac	tcc Ser ggc Gly cgc Arg 35 ggc Gly	gag Glu tcc Ser 20 ccc Pro	gac Asp 5 gtg Val tac Tyr ctg Leu aag	ds to 141  gtc Val  aac Asn  gag Glu  ccc Pro	atc Ile ggc Gly ggc Gly ttc Phe 55 tac	aag Lys cac His acc Thr 40 gcc Ala	gag Glu gag Glu 25 cag Gln tgg Trp	ttc Phe 10 ttc Phe acc Thr	atg Met gag Glu gcc Ala atc Ile	cgc Arg atc Ile aag Lys ctg Leu 60	ttc Phe gag Glu ctg Leu 45 tcc Ser	ggc Gly 30 aag Lys cct Pro	Val 15 gag Glu gtg Val cag Gln	ggc Gly acc Thr ttc Phe	96 144

5	aac Asn																336
	cag (																384
10	ccc Pro																432
15	tcc Ser 145																480
20	aag (																528
25	aag ( Lys '												. •		٠.		549
	RFP I					_									•		
30	(RFP SEQ			_			aa r	resid	lues	185-	-225	(end)	of	mRFI	?)		
	atg a Met 1																48
35	gac a																96
40	cgc q															·	126
45	KFP (KFP SEQ )	F1A	, cc	rres	pond	ds to		resi	dues	s 1-3	36 of	KFI	21)				
50	gcc ( Ala (																48
30	acc o																96
55	ccc (																105

5	(KF	P F2	A cor	rres	ded ( ponds & 10	s to		resid	dues	37-6	end (	of KI	FP1)				
10								atc Ile									48
			~				_	tcc Ser			_	_				_	96
15				_				ggc Gly 40			_			_	_		144
20								gag Glu									192
25			_		_		_	gac Asp			_	_		_	_	_	240
30								ggt Gly									288
00								cgc Arg									336
35								cgc Arg 120									384
40	_						_	acc Thr	_		_					_	432
45								ctg Leu									480
50								gag Glu									528
00	_	_			_	_		ggc Gly	_		_	_		-			576
55		ctg Leu															591

5	(KF	P F1	3 ČC	orre	Met spond & 10	ds to	oved o aa	res	idues	s 1-9	98 of	E KFI	P1)				
10							acc Thr										48
10							ttc Phe										96
15							gag Glu										144
20							cac His 55										192
25							tac Tyr										240
30							ttc Phe										288
	gac Asp		,														291
35	(KF)	P F21	3 co:	rresp	ded ( ponds & 10	s to	aa 1	resid	dues	99-6	en <b>d</b> o	of KI	FP1)				
40							gcc Ala										48
45							aag Lys										96
50							aag Lys										144
							ggc Gly 55										192
55	cta	aaσ	tac	ccc	qqc	ggc	cgg	cac	ctg	acc	tgc	cac	ctg	cac	acc	acc	240

5									ctg Leu 90						2	88
·									gag Glu							36
10									ggc Gly						3	84
15				ctg Leu											4	05
20	(KFI	P F10	C Co	s. 1 orres 1052	spond	ds to	resi	idues	s 1-1	153 (	of KI	FP1)				
25	_		_				_		ttc Phe 10	_				 		48
30									atc Ile							96
									atc Ile						1	44
35					-			_	tcc Ser			_	_		1	92
40									ggc Gly						2	40
45									gag Glu 90						2	88
50									gac Asp						3	36
30									ggt Gly						3	84
55									cgc Arg						4	32

5					gtg Val											456
10	(KF) SEQ	P F20	C co:	rres 1054	ded (pond)	s to 055	aa 1								000	48
														ggc Gly		40
15														aag Lys 30		96
20														cgc Arg		144
25														tac Tyr		192
30														ggc Gly		240
00	(KF	P F1	) c	orre	Met spond & 10	ds to		res:	idues	s 1-1	112 (	of KI	FP1)			
35														atc Ile		48
40														gag Glu 30		96
45														gag Glu		144
50														atg Met		192
50		_				_								tac Tyr	_	240
55																

5							gcc Ala										333
3	(KFE	P F21	) co	resp	ded ( pond: & 1	s to	s. 1 aa 1	resid	dues	113	-end	of I	KFP1	)			
10	_	_	-	_			aag Lys		_		_	~~					48
15							cag Gln										96
20							gtg Val										144
25			_	_	. –		ggc Gly 55				_		_		_	cac His	192
							aag Lys										240
30							cgc Arg										288
35	ggc						tac Tyr										336
40					_	_	ggc Gly									*	363
45	(KFF	F11	E CC	orres	Met Spond & 10	ds to	oved aa	resi	idues	3 1-1	169 (	of KI	FP1)				
50							acc Thr										48
50							ttc Phe									aac Asn	96
55							gag Glu										144

5										tcc Ser							192
Č										ggc Gly							240.
10										gag Glu 90							288
15										gac Asp							336
20	_	_			_		_		_	ggt Gly		Asn			_	_	384
25										cgc Arg							432
										cgc Arg							480
30							cgg Arg							٠			504
35																	
	(KFI	F2E, F2F ID N	CO	resp	onds	s to		resio	dues	170-	-end	of F	(FP1)	1			
40										tac Tyr 10							48
45										ttc Phe							96
50										tgc Cys							144
55										ccc Pro	Ser						192

KFP F1F, pos. 1 Met removed (KFP F1F corresponds to aa residues 1-186 of KFP1) SEQ ID NOS:1064 & 1065

5					atg Met			_			 	48
10					aag Lys							96
15					atg Met 40							144
20					atc Ile							192
					gtg Val						aag Lys 80	240
25					acc Thr							288
30		 	_	_	cac His	_	_		_	_	 _	336
35	tgc Cys				atc Ile 120							384
40					gtc Val							432
					gtg Val							480
45					cac His							528
50					tcc Ser							555

```
KFP F2F, Met added @ pos. 1
     KFP F2F corresponds to aa residues 187-end of KFP1)
     SEQ ID NOS:1066 & 1067
     atg ctg aag atg ccc ggc ttc cac ttc gag gac cac cgc atc gag atc
                                                                            48
     Met Leu Lys Met Pro Gly Phe His Phe Glu Asp His Arq Ile Glu Ile
                                         10
     atg gag gag gtg gag aag ggc aag tgc tac aag cag tac gag gcc gcc
                                                                            96
10
     Met Glu Glu Val Glu Lys Gly Lys Cys Tyr Lys Gln Tyr Glu Ala Ala
                 20
    gtg ggc cgc tac tgc gac gcc ccc tcc aag ctg ggc cac aac
                                                                           141
     Val Gly Arg Tyr Cys Asp Ala Ala Pro Ser Lys Leu Gly His Asn
15
             35
                                 40
     Unmutated fragments that form a part of the invention:
20
     ["aa" = amino acid]
    GFP F1: aa residues 1-39 of wt GFP (SEQ ID NO:2);
    GFP F2: aa residues 40-238 of wt GFP(SEQ ID NO:2);
25
    YFP F1A: aa residues 1-40 of EYFP(SEQ ID NO:4);
    YFP F2A: aa residues 41-239 of EYFP(SEQ ID NO:4);
    YFP F1B: aa residues 1-103 of EYFP(SEQ ID NO:4);
    YFP F2B: aa residues 104-239 of EYFP(SEQ ID NO:4);
    YFP F1C: aa residues 1-117 of EYFP(SEQ ID NO:4);
30
    YFP F2C: aa residues 118-239 of EYFP(SEQ ID NO:4);
    YFP F1DX: aa residues 1-158 of EYFP(SEQ ID NO:4);
    YFP F2DX: aa residues 159-239 of EYFP(SEQ ID NO:4);
    YFP F1D: aa residues 1-159 of EYFP(SEQ ID NO:4);
    YFP F2D: aa residues 160-239 of EYFP(SEQ ID NO:4);
    YFP F1E: aa residues 1-174 of EYFP(SEQ ID NO:4);
    YFP F2E: aa residues 175-239 of EYFP(SEQ ID NO:4);
    YFP F1F: aa residues 1-191 of EYFP(SEQ ID NO:4);
    YFP F2F: aa residues 192-239 of EYFP(SEQ ID NO:4);
40
    EGFPF2A: aa residues 41-239 of EGFP (SEO ID NO:3);
    RFP F1A: aa residues 1-39 of mRFP(SEQ ID NOS:15 & 16);
    RFP F2A: aa residues 40-225 of mRFP(SEQ ID NOS:15 & 16);
    RFP F1B: aa residues 1-101 of mRFP(SEQ ID NOS:15 & 16);
45
    RFP F2B: aa residues 102-225 of mRFP(SEQ ID NOS:15 & 16);
    RFP F1C: aa residues 1-115 of mRFP(SEQ ID NOS:15 & 16);
    RFP F2C: aa residues 116-225 of mRFP(SEQ ID NOS:15 & 16);
    RFP F1D: aa residues 1-153 of mRFP(SEQ ID NOS:15 & 16);
    RFP F2D: aa residues 154-225 of mRFP(SEQ ID NOS:15 & 16);
50
    RFP F1E: aa residues 1-169 of mRFP(SEQ ID NOS:15 & 16);
    RFP F2E: aa residues 170-225 of mRFP(SEQ ID NOS:15 & 16);
    RFP F1F: aa residues 1-184 of mRFP(SEQ ID NOS:15 & 16);
    RFP F2F: aa residues 185-225 of mRFP(SEQ ID NOS:15 & 16);
55
    KFP F1A: aa residues 1-36 of KFP1(SEQ ID NOS:17 & 18);
    KFP F2A: aa residues 37-232 of KFP1(SEQ ID NOS:17 & 18);
    KFP F1B: aa residues 1-98 of KFP1(SEQ ID NOS:17 & 18);
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KFP F2B: aa residues 99-232 of KFP1(SEQ ID NOS:17 & 18);
KFP F1C: aa residues 1-153 of KFP1(SEQ ID NOS:17 & 18);
KFP F2C: aa residues 154-232 of KFP1(SEQ ID NOS:17 & 18);
KFP F1D: aa residues 1-112 of KFP1(SEQ ID NOS:17 & 18);
KFP F2D: aa residues 113-232 of KFP1(SEQ ID NOS:17 & 18);
KFP F1E: aa residues 1-169 of KFP1(SEQ ID NOS:17 & 18);
KFP F2E: aa residues 170-232 of KFP1(SEQ ID NOS:17 & 18);
KFP F1F: aa residues 1-186 of KFP1(SEQ ID NOS:17 & 18);
KFP F2F: aa residues 187-232 of KFP1(SEQ ID NOS:17 & 18);
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While the many forms of the invention herein disclosed constitute presently preferred embodiments, many others are possible and further details of the preferred embodiments and other possible embodiments are not to be construed as limitations. It is understood that the terms used herein are merely descriptive rather than limiting and that various changes many equivalents may be made without departing from the spirit or scope of the claimed invention.